MAJOR HISTOCOMPATIBILITY COMPLEX OF THE DOG

PROEFSCHRIFT

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So instinctive is each one's conviction that he is unique in the world and privileged, that he feels that, however wrong it might be for others, what he for his part does, if not natural and right, is at least venial.

W. Somerset Maugham, 1938
The Summing Up, XVII

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CHAPTER I

INTRODUCTION

1.1 SHORT DEFINITION OF RATIONALE OF THESIS

Nowadays everybody has become familair with the procedure of blood transfusion. It is not always appreciated, however, that transfusions are equivalent to transplantations. This latter term is commonly reserved for the more spectacular transplantations of kidney, liver or heart, in which an organ from a deceased individual is given to a living human patient in need of such an organ.
It has become generally accepted that before blood of a given donor can be given to a particular patient investigations have to be done to exclude a possible harmful effect of the blood on the recipient. The impressive bloodbanking institutes all over the world are in themselves convincing examples of the importance of finding compatible blood for each individual patient. In theory compatibility tests between donor and recipient would also seem to be indicated for solid organ transplantations just as for tranfusions. In practice compatibility tests (or in other words donor selection) are not performed by all teams involved in transplantation in humans. Major reasons for this negative attitude towards donor selection for organ grafting are probably that 1) it appears to be a complicated art to the uninitiated and 2) no clear cut beneficial effect of donor selection could be demonstrated in some of the first clinical trials with human kidney transplantation (47, 53). However, in a number of other studies a favourable effect of donor selection has been found (28, 57, 94).
The experimental studies described in this thesis were initiated with the purpose of obtaining a model in an outbred experimental animal, in which the doubts on the usefulness of donor selection for organ transplantation could be evaluated.

1.1.1 Definition of histocompatibility

Donor selection for organ transplantation is necessarily a study of genetics.

This statement can be illustrated by a comparison of the survival of organs from donors, which differ in their genetic relationship with the recipient.
If inbreeding is pursued for a sufficient number of generations, a population of individuals will be produced which are genetically speaking identical. This has been achieved in so-called "inbred strains" of mice and rats. Identical (or monozygotic) human twins are another example of genetically speaking identical individuals. Tissue transplanted between members of a inbred strain or between human identical twins will survive as long as the recipient.
In other host donor combinations, where genetic differences *do* exist, the survival of a transplanted organ appears to be limited. It will initially be accepted, but gradually the recipient will by means of its immune system recognize the transplant as "foreign" and mount an attack against the donor tissue. This attack damages the transplant, which will loose its function and necrotize. The donor tissue is then said to be "rejected" by the recipient. The difference in fate between a transplant from a monozygote twin and one from an unrelated individual demonstrates that the ultimate success or failure ("rejection") of a tissue graft is determined by differences in genetically controlled structures in donor and recipient. The genes controlling these structures have been called histocompatibility genes, since they determine whether *tissue* (*histos* in Greek) will be *compatible* with a given recipient.

1.1.2 Rationale of choice of experimental animal

Besides the genetic control of coat colours (chapter 4, reference 15) relatively little is known of dog's genetics in comparison to the available knowledge in other domestic animals. The dog was nevertheless chosen as experimental animal for a genetic subject as donor selection because it is readily available, has a size attractive for transplantation surgeons and a temperament, which is suitable for an intensive post operative care. The dog has in fact been used in many transplantation experiments in the past but only rarely attempts have been made to define histocompatibility genes in this species (24, 25, 34, 37, 97).
Another advantage of dogs is that mongrel dogs and also purely bred dogs (91a) can be considered to be outbred, which is a property in common with human populations. This will facilitate an extrapolation of experimentally obtained results to humans. The best model for this purpose is of course the outbred non human primate and in particular the chimpanzee. Extensive use of this species in the laboratory is, however, limited by the fear of extinction of the chimpanzee and by the biotechnical and financial problems involved. Among primates Rhesus monkeys are the most commonly used experimental animals and extensive studies of donor selection have been performed in this species (e.g. 10). However, the shorter generation and gestation time of dogs offer better possibilities for family studies, which are an essential part of any genetic study.

Besides the interest in the role of genetics (or in other words histocompatibility) in the determination of organ graft survival, investigations have been started in several mammalian species to define other effects of histocompatibility structures. As will be discussed later (see 1.3.3), some preliminary evidence has been obtained that histocompatibility structures play a rôle in the determination of disease susceptibility. The dog might also be a useful experimental animal for this aspect of histocompatibility studies. It was probably the first animal to be domesticated by man (around 10,000 years B.C. (see (3) for references). The close physical proximity of man and dog from that time onwards made them share habitat, diet and exposure to other pathogens. Many human diseases occur also in dogs (chapter 5, reference 6). These diseases are ideal models for an experimental evaluation of a possible correlation between histocompatibility structures and disease susceptibility in dogs, since the obtained results might be very relevant for human patients, with the equivalent disease.

1.1.3 Lay out of contents of this thesis

The current use of histocompatibility testing in experimental biology and in human medicine can be seen as the logical sequel of advances in two different fields of research during the 20th century. A short survey of developments in both fields, i.e. 1) transplantation of tumour cells and 2) the serological determination of individual differences, will be given in part 1.2 of this chapter. Part 1.3 classifies current topics in histocompatibility research. It also serves as an introduction to chapters 2-5 which represent work already published in scientific journals. They are reprinted in chronological order illustrating the successive steps taken in the past few years to gain more knowledge on canine histocompatibility. Chapter 6 is devoted to a discussion of the obtained results. In this chapter the conclusions which were reached on the basis of the described studies are listed. An appendix describing the micro-cytotoxicity test used, a summary in English and Dutch, acknowledgements and a curriculum vitae of the author constitute the last part of this thesis.

1.2 HISTORICAL SURVEY OF HISTOCOMPATIBILITY

A survey of important developments of the past in the fields of tumour transplantation and immunogenetics gives an insight into the "why's" of histocompatibility. Both fields developed separately, but merged, when it appeared useful to determine prospectively differences between donor and recipient by serological methods. Only those aspects will be discussed here, which are relevant to the investigations described in the following chapters.

1.2.1 Transplantation of tumour cells

Origin of interest in tumour transplantation

M. Novinsky was probably the first (1876) to report a successful transplant of a piece of malignant tumour between individuals of one species. Incidently his experiments were performed with a medullary carcinoma in dogs (83). The interest of research workers in the beginning of this century in this type of experiments stemmed from their desire to prove or disprove the communicability of malignant growth. Soon they noted that a successful "take" and subsequent growth of transplanted tumour material was not regularly achieved. Viable tumour cells seemed to be a prerequisite for a successful transplantation. The success rate, however, appeared to be very unpredictable when outbred animals were used. In mice, which were inbred for several generations, more consistent success or failure rates were achieved with tumour cell injections (54). The question whether cancer must be regarded as a special kind of infectious disease will not be further dealt with here, since it is outside the scope of this thesis.

Genetic studies of tumor graft survival

In 1916 Little and Tyzzer (69) reported on experiments especially designed to study genetic influences on the growth of a transplanted tumour in mice. They concluded that the recipient's susceptibility to the tumour is controlled by mendelian systems located on several chromosomes. Another illustration of the influence of genetics on the occurence of malignancies is the difference in susceptibility to transplanted or spontaneously occuring tumours in the available laboratory mouse strains. The existing strains of inbred mice, differ in their genetic background and all have their own spectrum of susceptibility or resistence to different malignancies (81).

Comparison between transplantation of normal and malignant cells

Some of the genetical systems which influence susceptibility to injected tumour cells appear to be of equal importance for the survival of normal cells. Gorer (43) and Medewar and co-workers (75) have been able to show that the rejection or acceptance of foreign tissue is determined by the immunological reactivity of the host against parts of the cell membrane of these foreign cells. Most of these parts are found on tumour cell as well as on normal cell membranes and are thus not exclusively limited to tumour cells. However, tumour specific membrane parts have been found (for review of this subject see (58)). They might play a rôle in the genetic control of tumour susceptibility, since some

individuals might be able to react immunologically against these tumour specific structures and thus to destroy the tumour, while other might not. These individual differences in immunological reactivity might well be genetically determined, since examples of a genetic control of immunological reactivity have been found (72). There is ample evidence for the involvement of the immune system of an individual in the determination of tumour susceptibility. The most striking examples are the higher frequency of malignancies in immunosuppressed animals (77) and human patients with iatrogenic or congenital immunodeficiences (41, 74). A rapid further elucidation of this rôle of the immune system and the possible genetic factors behind it is evidently of great importance. Some of the existing theories on the possible rôle of histocompatibility in the genetic control of disease susceptibility will be discussed in the final paragraph of this chapter.

Those membrane parts, which normal and tumour cells have in common and which determine the survival of these cells, when transplanted to another individual, have already been introduced in the first paragraph of this chapter, under the name of histocompatibility structures. The genetic control of these structures have primarily been evaluated in transplantation experiments with murine tumours. In the following paragraphs the results of these studies will be briefly summarized with the major emphasis on two points, 1) the number of different genetic systems which control histocompatibility structures and 2) the relative importance or strength of such structures. For detailed information and further references on these matters the reader is advised to consult the review of Snell and Stimpfling (101).

The number of histocompatibility systems

In the last decades most transplantation experiments using normal or malignant tissue have been performed in and between inbred strains of mice. Other mammalian species without the economical advantages of small rodents have been less well investigated. However, even in mice the overall picture of genes controlling graft survival is far from complete. Striking similarities in mammalian histocompatibility systems (H-systems) have been found so far (see fig. 6.3). This warrants a further discussion of the available knowledge in the mouse.

For a thorough analysis of the number of H-systems in a species a large number of offspring has to be raised from random individuals, following an especially designed pedigree. Numerous grafts exchanged between members of different generations have then to be done. A determination of the percentage of successful grafts in these experiments will allow an estimate of the number of H-systems present. Obviously economical reasons limit this approach to small animals with short generation times. Experiments performed by Snell in 1948

(100) demonstrated the existence of at least 13 H-systems located on 13 different chromosomes in mice. Later experiments (23) have indicated that this number is probably an underestimate.

The strengh of a histocompatibility system

A difference between H-systems in a species appears to be the barrier which genes from different H-systems oppose to a transplant. Some of the barriers are more easily overcome than others. If mouse strain systems differ for genes of one particular genetic system, the H-2 system, grafts exchanged between them are rapidly rejected. The rejection pattern is not easily influenced by immunosuppressive procedures. The H-2 system is said to be the "strongest" or major histocompatibility system in mice. Differences in other H-systems do not lead to rejection as fast as an H-2 difference and are easier to overcome by immunosuppression. These systems have been labelled "weak" or minor histocompatibility systems. Graff et al. (44) demonstrated, however, that differences in minor histocompatibility systems can cumulate and that many incompatibilities for non H-2 systems can give rise to rejection times almost as short as when an H-2 difference is involved.

Tissue distribution of histocompatibility systems

Major histocompatibility genes are expressed on all cells of an individual except perhaps on the red cells of some mammalian species (50, 80). Some but incomplete data are available on the tissue distribution of minor histocompatibility systems. Lance et al. (61a) have been able to show that in epidermal cells in mice genes are expressed of a system which was labelled SK. They are certainly not expressed in haemopoietic or lymphoid cell lines and presumably not in other tissues, besides epidermis. Differences in the SK system between donor and recipient cause rejection of skin grafts and have been identified as a possible reason for the more violent and faster rejections of H-2 compatible skin grafts, when compared to other H-2 compatible tissues e.g. kidneys (99). The SK system has been called a system, controlling differentiation antigens, since it is only expressed in cells, which have differentiated in a certain way (as epidermal cells). Other differentiation systems are known in mice, e.g. Ly, TL, and Θ. They are of great importance in studies of fundamental immunology but will not be discussed here, since they have not been shown to have a histocompatibility effect. The division of H-systems into one major and an as of yet undetermined number of minor histocompatibility systems has appeared to be realistic for all mammalian species so far investigated. Man (1), rhesus monkey (10, 40), rabbit (106), pig (107), dog (this thesis), rat (87) and guinea pig (109).
When the data given in 1.2.1 are briefly summarized, it is clear that the

transplantation of tumour cells demonstrated the existence of genetic structures on mammalian cells, which are also occuring on normal cells. These structures determine the survival time of these cells in another individual. The next paragraph will describe the gradual development of methods for an in vitro identification of the products of genetic systems, which have a major influence on graft survival.

1.2.2 Serological determination of individual differences

Around 1900 Landsteiner (62) started his classical studies on properties of human red cells, controlled by the ABO system. Antibodies against antigens of the ABO system were found to be present in sera of normal unimmunized adults. After Landsteiner's experiments with these so-called "naturally occuring" antibodies, experiments were started by him and others, in which immunizations with blood or blood components between individuals of the same or different species were carried out. As expected these immunizations were found to give rise to antibodies against the cells used for immunizations. More important was the observation that such antisera also reacted with some but not all of the cell samples of other individuals from the same species as the donor used for immunizations. Soon the hypothesis was formulated that the structure on the cell, which reacted with the antiserum, was genetically determined. Therefore genetic tests have been applied to the results obtained with the produced antisera. The next paragraph will discuss this kind of analysis briefly.

Population and family studies of immunogenetic systems. Application of laws of Mendel and Hardy Weinberg.

If one wants to determine whether a factor recognized by a particular antiserum is genetically determined, the occurence of this factor has to be studied in populations of unrelated individuals and in families. For the ease of the argument it will be assumed that the antiserum under study contains antibodies against one factor only, although this is the exception rather than the rule after immunizations in unselected donor-recipient pairs. The analysis of sera with antibodies against more than one factor is more complicated and will not be discussed here (see 1.3.1). If many different immunizations are done, antisera against one factor only, so called "monospecific" antisera will be produced in at least some of the recipients. If such sera recognize a factor which is controlled by a gene from *one* genetic system, other gene(s) must exist for the same system, since some of the individuals do not carry the factor (are negative with the produced antisera). The term polymorphism has been applied to those genetic systems in which at least two alternative forms or genes (allelomorphs or alleles

for short) exist. When it is assumed that a genetic system exists, which is located on a particular area (locus) of chromosome and that different (two or more) forms of genetical information (allelic genes) can to the exclusion of each other occupy that area, the law of Mendel will predict the behaviour of the alleles in families. In general a parent, which carries alleles A and B, of one polymorphic system, has an equal chance of 0.5 to pass on A or B to his offspring. In reference 14 of chapter 3 examples and methods are given to test family data for concordance with Mendel's law. The distribution of alleles in a population of unrelated individuals can be predicted by the law of Hardy-Weinberg (for examples see (68)). According to this law two alleles P and Q will under normal conditions be distributed in unrelated individuals following their relative frequencies (p and q, $p+q=1$). A p^2 fraction of individuals will have allele P in a double dose (= homozygous for P), a 2 pq fraction will have P and Q (= heterozygotes) and finally a q^2 fraction of all individuals will have Q in a double dose (= homozygotes for Q).

Thus the patterns found with antisera in families and populations can be tested for accordance with expectations based on the laws of Mendel and Hardy-Weinberg. Statistical procedures will measure the significance level of the found deviations from the expected ones. If non-significant levels (for example $p \geqslant 0.1$) are found, the studied antibodies recognize probably factors controlled by alleles of one genetic system. Numerous genetic systems with multiple alleles, controlling factors of erythrocytes, leucocytes, platelets and serum proteins have been discovered in many different species by the use of the methods just described. The specificity of the immune response was thus proven to be a very useful tool for the recognition of some genetic polymorphisms. Irwin was the first to use the term immunogenetics for this kind of research activity.
In summary the described approach indicates that for a polymorphic genetic system one can attempt to read the formula gene → factor (antigen) → antibody backwards, i.e. starting at the antibody end (51), after the production of appropriate antisera.

Use of serological techniques for histocompatibility testing

Landsteiner (63) in his Nobel lecture in 1931 clearly indicated the potential value of serologically determined differences between individuals as indices of compatibility for transplantation. The results obtained in clinical skingrafting were, however, still very unpredictable in those days. Gorer in 1938 (43) was the first to demonstrate successfully how to bridge the gap between the already known genetic control of histocompatibility and the ignorance of how to determine these genetical tracts in vitro. In serological tests with a rabbit anti mouse serum he found evidence for the existence of an antigen common to cells of a certain mouse tumour and erythrocytes of some mouse strains. The tumour

was rejected by animals lacking the antigen on their erythrocytes, while it grew in animals carrying the antigen. Thus *histocompatibility genes seemed to be amenable to analysis by the determination of their end products, antigens, on the cell membrane.* To avoid confusion it should be stressed that only some of the systems, which have been identified by immunogenetical methods, appear to have an effect on organgraft survival of genetically different donors. Many of the immunogenetic systems found do not have such a histocompatibility effect.
Furthermore these data underline the earlier made remark that some genetically controlled factors (antigens) influence the survival of grafted tumour cells and occur on tumour cells as well as on normal cells (as red cells in this case).

Following the work of Gorer, initially erythrocytes have been used as test cells in histocompatibility antigen (H-antigen) determinations in mice. With the appropriate antisera human H-antigens can be demonstrated on all nucleated cells, (including reticulocytes (50)), but not or only some of them on erythrocytes (80). Leucocytes or lymphocytes from peripheral blood have become the most popular cells for testing in larger species than mice, since these cells are just as easy to collect as red cells and have a high density of H-antigens on their membranes (4).
A practical problem in white cell typing has been found to be the number of reagents required. In erythrocyte serology the presence or absence of an antigen can usually be determined by the reaction patterns of one antiserum. All antisera recognizing the same antigen have essentially the same reactivity pattern. For largely unknown reasons this one to one relationship does commonly not prevail in serological tests with leucocytes. Here groups of antisera with similar reactivity patterns are needed to reach a decision on the absence or presence of an antigen on the cells of a particular individual. An antigen is only said to be present when the majority of the available antisera are positive (the antisera have only roughly identical patterns, "false" positive and "false" negative reactions often occur). A different nature of leucocyte antibodies or antigens or both could be the cause of this complexity. As will be indicated later, the identification of the culprit of this complexity has to wait till the results of studies of the metabolism and biochemical nature of leucocyte antigens become available (see paragraph 6.4.1).
An objective assessment of the presence of an antigen in a particular leucocyte suspension can be made with the help of a computer which will sort out the sera with common reactivity patterns and allocate the antigen recognized by such a group of sera, to those cells, which are positive with a preset number of the sera from the group, with the common reactivity pattern. Van Rood (93) initiated this approach. His discovery has provided the essential starting point for a host of investigations in the last decade on human histocompatibility antigens. Mammalian major histocompatibility systems were found to consist of many

different alleles, controlling as many antigens. The fact that several antisera are needed for the determination of one antigen combined with the fact that many different antigens are present, make the population studies of such systems too cumbersome for analysis by hand. Again a computerized analysis has made such studies possible.

1.3 CURRENT TOPICS IN HISTOCOMPATIBILITY

A short historical survey of histocompatibility has been given in 1.2. In part 1.3 of this introduction chapter, three important current topics in histocompatibility will be described briefly.

Identity between donor and recipient for major histocompatibility structures favours graft survival, but does not guarantee an indefinite absence of rejection. In all the outbred mammalian species investigated, rejection of transplants of donors identical for major histocompatibility structures *does* occur. Non identity for minor histocompatibility structures will in the long run give rise to rejection phenomena in these donor-recipient combinations. However, no detailed knowledge of these systems is available (with the exception of some systems in small rodents (60, 61, 101). Therefore the following discussion will be limited to major histocompatibility structures. As was pointed out in 1.2.2 histocompatibility structures have been investigated by serological methods initially. Later cellular methods have been introduced. Previously it was assumed that both types of methods recognize the same structures. It will be shown (1.3.2) that in several species this conviction is now outdated and contradicted. This has led to a change in nomenclature. The major histocompatibility system is now called a major histocompatibility complex, since it consists of at least three parts: a) and b) two different series of multiple alleles, which control antigens, who can be recognized serologically and c) a third system, which is closely linked to a and b and controls structures which determine reactivity in cellular tests.

The first topic to be described (1.3.1) i.e. serological testing of major histocompatibility antigens and the demonstration of the existence of a major histocompatibility complex is directly related to the investigations reported in chapters 2-5. It can be read as an introduction to these chapters. The second and third topic (1.3.2), cellular testing of histocompatibility and (1.3.3) the biological significance of histocompatibility, are the main subjects for future studies and have hardly been touched upon in the investigations made for this thesis. These topics are nevertheless discussed here to show the relations between the three lines of histocompatibility research and to give a more complete survey of the field.

1.3.1 Serological testing of major histocompatibility antigens. Demonstration of the existence of a major histocompatibility complex.

It will be shown in this section that the existence of one major histocompatibility complex has to be proven in studies of families. A priori it would seem logical to demonstrate first that a major histocompatibility complex exists and if this is proven to be the case, to start the analysis of the constituents of this complex in detail. It appears, however, that this approach is only useful in a species in which large families are readily available for investigation. In primates blood samples of large families are difficult to obtain, since these families are scarce (rhesus monkeys and chimpanzees) or the logistics of sampling are complicated (man). In these species the analysis of major histocompatibility structures has started "at the other end", i.e. at the population level. First antigens were defined in a population of unrelated individuals and then their inheritance was studied in families to see if they belonged to the same system or complex (8, 9, 93). In dogs the first mentioned approach (familystudies before population studies) is the most efficient one and has therefore been used in the studies to be described, since large dog families are common. Moreover the results of family studies can be put to good use for the production of more and better antisera (see 1.3.1a). An alternative to the demonstration of *one* major histocompatibility complex by family studies of antisera patterns is the use of cellular tests as mixed lymphocyte cultures between family members (e.g. chapter 6 reference 24). The question "does a major histocompatibility system exist?" can be answered by the taking of five different experimental steps.

a. production of antisera
b. typing of families
c. segregation analysis
d. transplantation of organs between prospectively typed family members
e. analysis of major histocompatibility antigens in unrelated individuals

These different steps will now be discussed in more detail.

Step a: *The production of antisera*

Two different approaches exist for the production of antisera which react with histocompatibility structures.

a. Immunizations carried out between members of different species ("Xeno" or hetero immunizations) (31)
b. Immunizations between individuals of the same species ("allo" or iso immunizations).

Approach (a) has the advantage that a species can be chosen for antibody production, which is practical for economical or other reasons. A drawback of "xeno" immunizations is that it is more difficult with this method to obtain antisera with a limited reactivity, e.g. versus one antigen only, than when "allo"

immunizations are used. It is, however, sometimes possible by elution and/or absorption procedures to refine a reagent obtained from xeno immunizations sufficiently. An advantage of xeno immunizations is however, that higher titers are obtained. (Zweibaum, personal communication). This is of course advantageous, since more individuals can be tested with a limited amount of antiserum which can be used in dilutions. High titers are also helpful in immunofluorescence or other tests, in which serum dilutions have to be used to exclude aspecific phenomena.

"Allo" immunizations have been used more often for the above mentioned reason of easier control of specificity of the products. Three approaches are available:

1. pregnancies (88, 92)
2. immunizations with whole blood, leucocytes of lymphocytes with or without Freunds adjuvans (8, 102)
3. a first set rejection of solid organ allografts.

The most commonly applied method in human tissue typing is the first one. Van Rood et al. and Payne et al. discovered independently that in approximately 15% of the sera of women, who had given birth to one or more children, antibodies against leucocytes of the father were present. The majority of these antibodies appeared to be directed against major histocompatibility antigens. The great advantage of this method is that no human voluteers are needed for the production of human typing reagents (approach 2). Incidentally naturally occuring immunization (i.e. pregnancy) does *not* seem to have an adverse effect on the survival of subsequent allografts, in contrast to the fate of allografts in multiple transfused patients who have developed antibodies against major histocompatibility antigens (13, 53, 85). Many "allo" antisera are made by approach 2. A situation in between xeno and allo immunizations is found in chimpanzees where allo chimp sera have also provided useful reagents for human tissue typing (9), as some of the leucocyte antigens of chimpanzees are almost identical to the human ones (30).

In dogs it has been our experience, that a skin graft is a very useful method for the production of high quality reagents (see chapter 2). This has also been documented for other experimental animals (8). It is important to keep the genetic difference between a donor and recipient small. This will limit the number of antigen differences between donor and recipient and consequently the number of different antibodies produced. Without any knowledge of major histocompatibility antigens the situation of limited differences can be achieved by grafting between parent and sib. Van der Does et al. (29) have reported on studies in which this approach was followed consequently.

Method of testing

The most commonly used test is the microcytotoxicity test in a one (56, and appendix) or two stage version (105). In this test the lymphocyte suspension to be tested is added to an antiserum. Complement is present (one stage version) or is added later (two stage version) after a period of incubation of cells and serum. Cells to which antibody and complement are fixed will be damaged. The damage can be visualized, since these damaged cells will take up dyes as trypane blue or eosine, while undamaged cells are not stained.
Other useful methods are the microcomplementfixation test (27) which has the advantage of easy antigen storage and rapidity or the fluorochromasia method (16). A good viability of cells before testing is a prerequisite. If too many cells, aspecifically damaged, take up dye, the effect of an antiserum can no longer be evaluated. In the fluorochromasia test, stored lymphocytes with an elevated number of dead cells can still be tested. Another advantage is that recently an automated test was developed (20). The choice of a particular test will depend on the available facilities and the personal preference and experience of the investigator. Changes from one test to another can be troublesome, since the number of individuals whose cells react with a particular antiserum, depends on the sensitivity of the test used. Tests with a higher sensitivity can increase the number of individuals positive with a particular serum and thus change the previous definition of the factor recognised by that serum.

Step b: *Typing of families*

Family studies can only be done, when the produced antisera indicate differences between family members or in other words "segregation" is found. A factor present in a parent is said to segregate in the offspring, when some of them obtain the factor and others do not. The chance of finding segregation is determined by the frequency of positive results of an antiserum in unrelated individuals (the so called population frequency of a serum). Sera with a low population frequency will often not react with either of the parents. Consequently no segregation will be found, since the whole offspring will be negative. Sera with high population frequencies will be often positive with cells of both parents and their whole offspring, so again no segregation of factors is found. Twelve sera or more with population frequencies of around 50% give a reasonable chance of obtaining segregation in every investigated family. These are thus minimum requirements for further studies.
A disadvantage of this approach is that antisera recognizing antigens with high population frequencies (as 4a/4b in humans (93)) are initially neglected. However in a later stage of the analysis of a histocompatibility complex a special search can be made for such antigens.

Step c: *Segregation analysis*

This analysis is performed on the basis of the following two assumptions.

1. one major histocompatibility complex (MHC) does exist in the species under investigation
2. the produced reagents contain antibodies against antigens controlled by this complex.

The consequences of these assumptions are illustrated in Tables 1, 1a and 1, 1b. Table 1. 1a shows the inheritance pattern in a hypothetical family. The paternal homologous chromosome pair, carrying the MHC information is labelled A/B. Each letter represents one member of this pair or a haploid part of the MHS information. Ceppellini (22) introduced the term haplotype for this notation. The maternal chromosome pair is indicated by the letters C/D. A maximum of four different possibilities exist for the offspring (in the notation used here AC, AD, BC and BD). Table 1. 1b lists all possible segregation patterns of the same family for sera recognizing major histocompatibility antigens.

With a limited amount of typing sera not all patterns listed in table 1. 1b will be found. With the above mentioned assumptions, however, missing patterns can often be deducted from those found. For example pattern 3, (the segregation of paternal chromosome A or haplotype A) has to be complementary to pattern 4 (the segregation of chromosome B). If one of the two patterns is missing, the missing one can be extrapolated from the one found. Particular families will not contribute to the segregation analysis, since by chance all the offspring obtained the same haplotypes, or parents are homozygous for the MHC or MHC haplotypes are shared by parents. In the typing of some families patterns might be found which are conflicting and cannot be explained by the two assumptions listed above.

For example antisera, which are positive for parent one and negative for parent two, should have the same segregation patterns or, when they differ, sibs can be positive for only one of the two. Sibs, double positive or negative for those reagents cannot be explained by the rules laid down in the two assumptions. (e.g. child 3 positive for serum 3 or negative for serum 4)

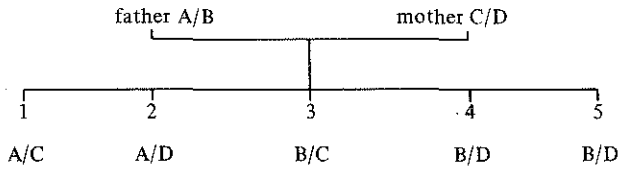
Sera showing these results should be disregarded for this analysis, but kept, since their antibodies might be directed against factors of other systems than the major histocompatibility complex.

One can try to avoid these conflicting results by using antisera from short immunization protocols (as a first set skin graft, see chapter 2). Prolonged immunization will lead to the emergence of "contaminating" antibodies against factors controlled by systems different from the MHC. The occasional occurrence of these results is perhaps best explained by serological errors, although crossing over as we shall see later (chapter 5) is also a possibility. Another explanation for

segregation patterns, conflicting with the two basic assumptions is an agglutination negative, absorption positive (ANAP) phenomenon (93, 112). In this case the presence of a factor can sometimes only be demonstrated by the performance of absorption tests. If a serum shows ANAP reactions, some false

Table 1.1

A. Segregation of parental homologous chromosomes carrying the information of *one* genetic system.



B. List of all possible segregation patterns, which can be found for this system with serological tests.

	family members and their haplotypes		SERA 1	2	3	4	5	6	7	8	9	10
	A/B	Father	–	+	+	+	–	–	+	+	+	+
C	C/D	Mother	–	+	–	–	+	+	+	+	+	+
E	A C	Child 1	–	+	+	–	+	–	–	+	+	+
L	A D	Child 2	–	+	+	–	–	+	+	–	+	+
L	BC	Child 3	–	+	–	+	+	–	+	+	–	+
S	B D	Child 4	–	+	–	+	–	+	+	+	+	–
	B D	Child 5	–	+	–	+	–	+	+	+	+	–
	"Haplotypes recognized"		–	ABCD or ABC or ABD or ACD or BCD	A	B	C	D	BD	BC	AD	AC

negatives will be found if only a direct, simple serological test is performed. Double negative sibs, as mentioned above could be explained in this way. Generally a "best guess" of MHC haplotypes can always be achieved in family studies, whenever conflicting segregation patterns are found, since the majority of sera can be presumed to give the proper inheritance of MHC haplotypes. The frequency of conflicting segregation patterns decreases to zero when optimal reagents have been selected after prolonged studies (own unpublished observations).

Segregation patterns and binomial distribution

The segregation pattern found can be expressed as a ratio with the more frequent chromosome as the first term. In the example of table 1.1b the ratios are B:A = 3:2 for the paternal chromosomes and D:C = 3:2 for the maternal chromosomes. It can be shown that the expectations of the possible different segregation ratios of a sibship size S are the sums of the symmetrical terms of the binomial distribution $(1/2 + 1/2)^S$ when only one homologous chromosome pair is involved in the determination of the genetical traits studied (71). These binomial coefficients can be found directly in a table of a part of the so called triangle of Pascal, which is given in table 1.2. Coefficients on one horizontal line, belong to the corresponding sibship S. The first and last coefficient of a line are always 1. The values of the others can be obtained by adding the two

Table 1.2

Part of triangle of Pascal.
List of coefficients of binomial distribution

S (sibsize)								
1				1				
2			1	2	1			
3		1	3	3	1			
4	1	4	6	4	1			
5	1	5	10	10	5	1		
6	1	6	15	20	15	6	1	
7	1	7	21	35	35	21	7	1
etc.								

coefficients, in the line directly above a particular coefficient. A sibship of 5 (S = 5) can have the following ratios 5:0, 4:1 and 3:2. These ratios have according to Pascal's triangle the following expected relative frequencies

$5{:}0 = \frac{(1+1)}{32} = 0.0625$, $4{:}1 = \frac{(5+5)}{32} = 0{,}3125$ and $3{:}2 = \frac{(10+10)}{32} = 0{,}6250$.

When a number of families has been typed, the difference between the observed and expected segregation ratios can be tested for significance in a statistical test. When a non significant deviation from expectations is found the two assumptions mentioned in the beginning of this paragraph can be accepted. Thus formal proof of the existence of a genetical system or complex on one chromosome pair can be obtained. This approach is documented for dog family studies in chapter 2. This approach cannot be used when selective pressures on the traits under study are causing differences in survival of sibs before the time of testing. Such an effect could not be demonstrated in human or dog families, typed for the major histocompatibility system (71, chapter 2).

Step d: *Transplantation of organs between prospectively typed individuals*

When step 1, 2, and 3 have successfully been performed, investigations can be started to determine the influence of the found genetic region on allograft survival. The survival times of grafts exchanged between siblings, which are identical for the system under investigation (e.g. children 4 and 5, (table 1.1b) have to be compared to the survival times of grafts from siblings who are *not* identical (children 2 and 3). If grafts in the former group survive significantly longer than grafts in the latter group, one can assume that the genetical region involved is a major histocompatibility complex (see chapters 2 and 4 for examples of survival times of allografts exchanged between sibling dogs).

Step e: *Analysis of major histocompatibility antigens in unrelated individuals*

Specificity of antisera.

A sufficient insight into the haplotypes of the MHC in family members can be obtained by reagents of limited quality, when the inheritance patterns are clear and the family members are only compared to each other. For the determination of genotypes in related or phenotypes in unrelated individuals a much more detailed knowledge is needed. For this second phase in the recognition of a histocompatibility complex many different antisera are needed with *low* frequencies of positive results in unrelated individuals ("low population frequency" sera). They can be obtained by the methods indicated (see 1.3.1 step a). Sera with high population frequencies commonly react against more than one specificity (antigen). Such sera are called polyspecific and their results are more difficult to analyse than those of monospecific sera. The number of different

antigens recognized by a serum can be investigated by absorption experiments (see chapter 2, reference 13). In tables 1.3 and 1.4 results are given of hypothetical absorption experiments with a monospecific and a polyspecific antiserum respectively. All the activity of monospecific antisera is removed by absorptions independent from the individual which is used as source for absorbing cells, as long as his cells react with the unabsorbed antiserum.
The activity of polyspecific sera will not be completely removed by all the cells used for absorption, since some do not carry all the antigens recognized by the sera (table 1.4, cells 2 and 3).
Absorption experiments are not the final answer towards the question of

Table 1.3
Reactions of unabsorbed and absorbed "mono specific" antiserum.

		SERUM					
		unabsorbed	absorbed with cell				
			1	2	3	4	5
CELLS	1	+	-	-	-	-	+
	2	+	-	-	-	-	+
	3	+	-	-	-	-	+
	4	+	-	-	-	-	+
	5	-	-	-	-	-	-

Table 1.4
Reactions of unabsorbed and absorbed "poly specific" antiserum.

		SERUM					
		unabsorbed	absorbed with cell				
			1	2	3	4	5
CELLS	1	+	-	-	+	-	+
	2	+	-	-	+	-	+
	3	+	-	+	-	-	+
	4	+	-	+	-	-	+
	5	-	-	-	-	-	-

polyspecificity. Some sera will be monospecific in absorption experiments, but recognize nevertheless more than one antigen. This occurs when an antiserum is not specific enough to recognize one single antigen. Through its "broader" specificity such an antiserum will react with several antigens, which have small differences, but also many similarities. This phenomenon has been labelled cross-reactivity (104). It is a complicating factor in an immunogenetical analysis and will be further discussed in 6.4.1.
Since monospecificity of antisera is difficult to prove, most population studies with antisera against histocompatibility antigens are done with crude sera, whose poly or monospecificity is not determined. If only those sera are included in a population analysis, which have a low population frequency, evidently the chances of working with monospecific sera are increased, which reduces the complexity of the data analysis.

Computeranalysis

It was shown by Van Rood (93), that the reactivity patterns of many antisera in large groups of cells of unrelated individuals can be analysed most efficiently with the help of a computer. If this machine is properly instructed it will group sera with similar reactivity patterns. The rest of the analysis is based on the assumption that sera with similar reactivity patterns recognize one factor (or antigen). The genetic and statistical tests which can be applied to investigate the relationships between these factors are discussed in the method sections of chapters 3 and 5. When enough antisera with low population frequencies have been produced and analysed, all or practically all factors belonging to a complex will be recognized in a population of unrelated individuals and complete or almost complete phenotypes of these individuals can be determined. Experiments can then be initiated to test the influence of the complex on allograft survival in unrelated donor/recipient pairs.

Transplantation of organs between prospectively typed unrelated individuals

In all mammalian species, investigated so far, serological tests have been successful in selecting good donors for organ transplantations from a group of family members. In humans it has not been possible to document the same beneficial effect of donor selection by serological methods in unrelated donor-recipient pairs. Nevertheless in some studies (28, 57, 94) a favourable effect could be demonstrated, it was however not as strong as in related donor/recipient pairs. This discrepancy will be further discussed in 6.6. It will be shown there that the explanation or a part of it might be found in a better definition of histocompatibility structures, recognized by cellular methods. These methods will be discussed in the next part of this chapter 1.3.2.

1.3.2 Cellular testing of histocompatibility

Mixed lymphocyte cultures (M.L.C.'s)

When lymphocytes from two normal, but genetically dissimilar individuals from the same mammalian species are mixed and cultured under the right experimental conditions for a number of days, a substantial number of the cells are stimulated to transform and start to divide. The incorporation of H3 or C14 labelled thymidine in the newly formed DNA can be used as a quantitative measure of this mitotic activity (5, 7). Initially it was thought that the thymidine uptake was mainly influenced by differences in serologically defined major histocompatibility antigens of the donors of the two lymphocyte populations. In general sibs, identical for these antigens did not stimulate each others lymphocytes in mixed lymphocyte cultures. Recently, however, it became evident through studies in families or strains in which crossing over within the major histocompatibility complex had occurred, that in several species, mouse (6), man (32), rhesus monkey (10), and probably dog (see chapter 5) M.L.C. reactivity is not governed by the Serological Defined antigens (S.D. antigens) but by at least one other genetic system, closely linked to the systems recognizing S.D. antigens. This part of the major histocompatibility complex has been labelled L.D. (or Lymphocyte Defined). Some investigators (26, 47, 95) have claimed a better correlation between serological typing and graft survival of unrelated donors when M.L.C. reactivity was also taken into account. The question of which part (S.D. or L.D. part) of the M.H.C. is more important or whether both play a rôle in graft survival is still unsolved. Van Rood (96) has recently proposed an attractive hypothesis on the basis of data obtained in the analysis of human kidney transplantation from unrelated (cadaver) donors (53) and from Eijsvogel's et al. (33) in vitro experiments. In his view both parts of the M.H.C. are important. Differences in L.D. structures between donor and recipient would trigger (or sensitize) the recipient. The S.D. structures of the donor would provide the targets for the immune response of the sensitized recipient. This theory awaits further proof and will be further discussed in chapter 5 and 6.

Besides the enormous experimental applications of the M.L.C. test, it has to be kept in mind that it has the great disadvantage of taking approximately a week before an answer is obtained. Therefore, it is unsuitable for a prognostic evaluation of histocompatibility when cadaver donors are used. In organ transplantations current organ preservation methods routinely allow no more than 36-72 hours preservation (14) of an isolated organ. Thus the transplant has to be performed before the results of MLC tests between donor and recipient are known.

Another limitation of MLC studies used to be that no systematical approach was available to study differences in L.D. structures between unrelated individuals. An ordinary MLC test between two unrelated individuals does not allow a precise identification of the L.D. structures of these individuals. A new approach (76), which has been successful in achieving the definition of some L.D. genotypes and phenotypes, is mentioned in chapter 5. It consists of the use of cells of individuals who are homozygous for their L.D. region of the M.H.C. Other new promising approaches are given in a recent paper by Van Leeuwen and Van Rood (64). In the past mixed lymphocyte cultures have been difficult to perform in dogs (see chapter 5). Recently a modified technique has given better results (Van den Tweel, personal communication).
The investigations performed for this thesis have mainly dealt with the S.D. part of the canine major histocompatibility complex. In 6.6 some, more recent, results, which were obtained in collaboration with Van den Tweel and coworkers and Grosse Wilde and coworkers on canine L.D. structures will be given.

Other cellular tests

In the past three methods besides the MLC test, have been used for a "cellular" determination of histocompatibility. These tests are known under the names of

a. normal lymphocyte transfer (NLT) (18)
b. third party test (TPT) (70, 111)
c. irradiated hamster test (IHT) (91)

None of these tests is used now very frequently. For technical details the reader is advised to consult the appropriate reference. All tests take at least 48 hours, cannot directly be interpreted in genetical terms (phenotypes or genotypes), give only a rough indication whether a given host-donor combination is good or bad, and are only mentioned here for completeness' sake. NLT and TPT have the additional disadvantage of requiring the cooperation of human volunteers, who will become sensitized to a considerable number of histocompatibility antigens.

1.3.3 Biological significance of histocompatibility

The majority of experimental histocompatibility studies have been oriented towards the exploration of transplantation immunology. In this part the more recent trend to demonstrate a biological function of major histocompatibility systems will be illustrated and discussed. Parts of cell membranes, which are amenable to analysis because of their antigenic properties, such as histocompatibility antigens or blood groups, have astonished scientists by their many forms. Individual variation or polymorphism of genetic systems appeared to be

greater than was generally expected. The polymorphic features of genetic systems, controlling cell surface antigens have in the past been considered as clear evidence of a rôle for all these systems in natural selection ("Pan-selectionism").

Pan-selectionism

Falconer (36) expressed the "pan-selectionism" view as follows: "There can be little doubt that the existence of genetic variation is advantageous to the evolutionary survival of a species. The advantage it confers being the ability to evolve rapidly and so to meet the needs of a changing environment, both through the course of time and in the colonisation of new localities ( ). The evolutionary significance of genetic variability, however, throws no light on the mechanism to maintain it".

E.B. Ford (39) gave examples of polymorphisms occuring in nature with a clearcut rôle in the determination of the "fitness" of an animal. He divided the polymorphisms into two groups.

1. *transient*, in which a new gene which possesses and preserves an overall advantage over its alleles spreads through the population by this selection advantage, until the other alleles are reduced to the status of a rare mutant.
2. *balanced*, in which the polymorphism that is produced by evolutionary changes in a species, comes to a lasting equilibrium after a short period of changing gene frequencies.

Several mechanisms for maintaining polymorphisms of type 2 in balance have been documented by Ford (39) and others (110). The one which was most regularly assumed to be responsible for doing so, was the so called "over dominance" or "heterozygote advantage" mechanism in which the heterozygote of a certain system (e.g. A/B) is at an advantage in natural selection, when compared to both homozygotes (A/A and B/B). Two critisisms of the "pan-selectionism" view have been voiced (49).

1) In several species 30% of the total of genetic information of the species is estimated to be polymorphic (48, 67). On theoretical grounds only a limited number of these polymorphisms can be balanced and maintained by heterozygote advantage. If all the polymorphic systems would show heterozygote advantage, many homozygotes would be conceived, who are "genetically speaking" less fit than the heterozygotes. A difference in fitness can be expressed in natural selection in two ways. Less fit individuals die early or produce no or decreased numbers of offspring. The observed mortality and fertility in primitive human populations where selectional forces are unhindered in their expression are not in accordance with expectations based on 30% polymorphic genetic systems with heterozygote advantage (21).

2) Another argument stems from the work of Kimura (55) who, on the basis of

a mathematical analysis of the evolution rate of proteins, declares that the majority of genetical polymorphisms can be causally explained by random changes of gene frequencies. This would add a third type of polymorphism to the two classical ones, described by Ford (1) transient and 2) balanced) i.e. 3) neutral. Such a polymorphism is also in a transient state, but changes in gene frequencies of the alleles of the system are not directed by natural selection, but fluctuate through the cause of other genetic mechanisms such as genetic drift, migration or mutation (36). Recently reviews of the two opposing theories of the significance of polymorphism "pan-selectionism" versus neutrality have appeared (21, 49). The interested reader is referred to these articles for details. Only a summary of the conclusions reached is given here. The most commonly held view now is, that much less genetical variation is significant in terms of natural selection, than was formerly thought to be the case. However, well investigated human polymorphisms with biological significance have been documented (e.g. sickle cell trait, G6PD variations and rhesus blood group system). Major histocompatibility complexes have not yet been definitely classified as belonging to either the "neutral" (type 3) or the "selection" polymorphisms (types 1 or 2). The available evidence on the possible rôle of the complex in natural selection is discussed below.

Histocompatibility and natural selection

A direct search for the influence of natural selection on M.H.C. products is impossible since the exact function of these structures is still unknown. This makes it necessary to use indirect methods to solve this question of a possible biological significance of histocompatibility structures. These methods have been divided into three useful categories for blood group polymorphisms by Gershowitz and Neel (42). The same classifications can be used for a description of indirect attempts to find evidence for a selection value of major histocompatibility antigens i.e.

1. associations between diseases and certain histocompatibility antigens.
2. mathematical formulae from population genetics can predict the behaviour of alleles and their frequencies in different populations under different circumstances. Selection, if present, has an effect on gene frequencies different from other genetic processes such as genetic drift, mutation and migration.
3. association between histocompatibility antigens and parameters of biological fitness (e.g. longevity, birth weight, reproductive potential etc.).

Diseases and histocompatibility antigens

Examples of category 1) have been found in two different species man and

dog (chapter 4). Human diseases in which a more frequent occurrence of certain histocompatibility antigens has been found in patients in comparison to a not afflicted control group, include morbus hodgkin (2, 79), glomerulonephritis (86), multiple sclerosis (82), lupus erythemathodes (45) coeliac disease (35, 103), chronic active hepatitis (73), ankylosing spondylitis (19, 98) and myasthenia gravis (90). The strongest correlations between a particular S.D. antigen and disease have been found in the last four examples. This could indicate a different relationship between disease and antigens in these cases (see paragraph on genetic control of disease susceptibility).
In disease association studies two artefacts can occur. The first one is the statistical problem of making many comparisons. This enhances the chance of finding improbable events (such as a very uneven distribution of a factor in two different populations). When many antigens are investigated in patients and normal subjects, some antigens will be abnormally distributed. The observed p values of the differences in antigen frequencies in the two populations have to be seen in this light and have to be corrected for the number of comparisons made (45). A second artefact is that an association between a particular disease and antigen might in fact be caused by a causative relationship between the disease and a genetic trait, controlled by a gene very closely linked to the allele controlling the S.D. antigen and in linkage disequilibrium with this allele (see next paragraph for description of linkage disequilibrium).

Population genetics and histocompatibility

Studies in category (2) of Gershowitz and Neel have been less frequent. The best example is the fifth human histocompatibility workshop, in which samples of populations from all over the world were studied for major histocompatibility antigens and other genetic polymorphisms. For details of the mathematical tests and methods used to test whether human major histocompatibility antigens are under selective pressure, the reader is referred to the book of Cavalli-Sforza and Bodmer "The Genetics of Human Populations" (21). The analysis of the data obtained at the workshop, indicated that major histocompatibility antigens are indeed significant in terms of natural selection (17). Earlier studies by Bodmer and Bodmer (15) and Piazza et al. (89) allowed the same conclusion.

Another possible example of selectional forces connected to histocompatibility is the occurrence in humans of linkage disequilibrium between major histocompatibility antigens of different, but closely linked series of multiple alleles (1). Generally genes from different systems on the same chromosome are not associated in a population of random individuals (see Li's illustrating example of the glove club in "Human Genetics" (68)). If antigens of different, but linked, series of multiple alleles are found more often in the same individual than would be

expected by chance alone linkage disequilibrium is said to be present between the alleles of the two series. Evidence for linkage disequilibrium between major histocompatibility antigens (S.D. antigens) has been obtained in humans (1), dogs (chapter 4) and more recently also in rhesus monkeys (12a) and chimpanzees (12). One of the possible explanations for this phenomenon is the existence of selection pressure favouring special antigen combinations. As mentioned earlier a genetic system governing reactivity in mixed lymphocyte cultures, the L.D. part of the M.H.C. (see also chapter 5 and 6), is closely linked to the systems controlling serologically defined antigens (or S.D. part of the M.H.C.) in man; mice, rhesus monkeys and probably dogs. In populations of humans, rhesus monkeys and dogs negative M.L.C. reactions do occur more often in S.D. identical unrelated individuals, than in unrelated individuals, differing for these antigens (see 6.6). This indicates the existence of linkage disequilibrium between S.D. and L.D. parts of the major histocompatibility complex (M.H.C.) in these species. The demonstration of linkage disequilibrium between three closely linked loci, the two S.D. loci and the L.D. locus could be interpreted as being evidence for a chromosomal region, which is carrying a so called "supergene". R.A. Fischer, who introduced this term, showed (38) that genes, which interact, can be kept in close association (notwithstanding the occurrence of crossing over), when only small selectional forces are working on them. Thus the data obtained in population genetics do allow the presumption that the L.D. and the two S.D. parts of the M.H.C. are kept together by selectional forces and therefore that the M.H.C. has a biological function. They do not give an indication towards the nature of this biological function. Finally the striking similarities between the M.H.C. of different mammalian species (see figure 6.3) could be another argument for the biological significance of this complex. Perhaps a greater divergence in present day mammals from the M.H.C. model of their common evolutionary ancestor would have been found if no selective pressures had been connected with this genetic region.

Histocompatibility antigens and parameters of biological fitness

This title was given to the third category of the earlier mentioned classification of Gerskowitz and Neel, who subdivided investigations into the possible biological significance of polymorphic genetic systems.
In mice (72), guinea pigs (65), rats (46), and rhesus monkeys (11) evidence has been obtained for the existence of a genetic control of the immune response. By the use of simple antigens for immunizations (e.g. synthetic polymers of a limited number of animo acids) genes were found, which controlled the ability of an individual to react with antibody formation against the injected antigen. The immunological response to each antigen investigated so far is probably controlled by a separate genetic system (11). Genes belonging to these systems

have been called Immune response or Ir genes.
Most of these genes appear to be linked to the major histocompatibility complex of a species. Under the heading of Disease and Histocompatibility Antigens, several human diseases were enumerated, which showed a positive correlation with a certain major histocompatibility antigen. If linkage disequilibrium can be proven to exist between Ir genes and major histocompatibility antigens, a reasonable explanation for the increased frequencies of such antigens among patients, with certain diseases can be given. Antibody formation against and thus susceptibility towards the causative factor of the disease might be controlled by genes of an Ir system.
The found correlation between diseases and major histocompatibility S.D. antigens is then only an artefact (as already discussed in the paragraph on disease and histocompatibility antigens), which is created by the linkage disequilibrium between Ir genes and the particular S.D. antigen. Thus the major histocompatibility complex might be relevant to important immunological functions of an individual if the "supergene" of the major histocompatibility complex might not be limited to two genes of the S.D. series and one gene of the L.D. series, but would also consist of an undetermined number of Immune Response genes.

Genetic control of disease susceptibility

Theories about a genetic control of disease susceptibility are manifold. Three of them will be discussed here since they can be related to the subject of histocompatibility. The first theory has been labelled "mimicry". It assumes that a pathological agent is crossreacting with a structure in the patient itself. So if an infective or oncogenic agent is very like a "self" component of some individuals in a species, those individuals will be more susceptible to these agents, since they will not raise an efficient immune response to them. Two possible examples of mimicry have been reported: 1) Vogel et al. (108) have demonstrated a cross reactivity between Pasteurella pestis and vaccinia with certain ABO blood group substances. Their results could not, however, be reproduced by others. 2) Hirata et al. and Mittal et al. (52, 78) have found similarities between bacterial products and human MHC antigens. A second theory could be named "receptor theory", in which a serologically recognized membrane structure is also give a receptor function for a pathological agent. Some of the diseases in humans with an almost 100% relationship between disease and M.H.C. antigens could be examples of this theory (coeliac disease etc., see earlier paragraph about diseases and histocompatibility antigens). At the moment no experimental data are available to prove or disprove this concept. The third theory has already been discussed partially in the previous paragraph. It links the susceptibility to a disease to the capacity of an individual to produce antibodies to the causative agent. Thus Ir genes of an individual determine whether he will be able to resist a certain

pathogen or not. Experiments performed by Lilly et al. (68a) have shown that suceptibility to an experimental induced leukaemia in mice is determined by genes from or linked to the major histocompatibility complex of this species. This might be an example of the action of Ir genes. Finally two examples will be given of diseases in which the circle genetic control of immune response, disease susceptibility and histocompatibility has been closed.
In choriomeningitis in mice (84) and in hay fever in man (66) the immune response to an agent, which is not pathogenic on its own, appears to be related to the presence of the disease. In both cases linkage between the major histocompatibility complex and the genetic control of the immune response to the agent has been found.

Since the first genetic studies of Little and Tyzzer (69) of malignant cell transplants several decades of intensive research activities on the genetic control of disease suceptibility have passed. Recently it appeared that the major histocompatibility complex might be important in this respect. Further histocompatibility studies in this direction are evidently of great importance. It has provided an additional rationale for the studies to be described in the following chapters.

1.4 REFERENCES

1. Allen, F., Amos, D.B., Batchelor, R., Bodmer, W., Ceppellini, R., Dausset, J., Engelfriet, C., Jeannet, M., Kissmeyer-Nielsen, F., Morris, P., Payne, R., Terasaki, P., Rood, J.J. van, Walford, R., Zmijewski, C., Albert, E., Mattiuz, P., Mickey, M.R. and Piazza, P. 1970. Joint report of the fourth international Histocompatibility Workshop. *In*: Terasaki, P.I. (ed.). Histocompatibility Testing 1970, p. 17. Munksgaard, Copenhagen.
2. Amiel, J.L. 1967. Study of the leukocyte phenotypes in Hodgkin's disease. *In*: Curtoni, E.S., Mattiuz, P.L. and Tosi, R.M. (eds.) Histocompatibility Testing 1967, p. 79. Munksgaard, Copenhagen.
3. Angress, S. and Reed, C.A.B. 1962. An annotated bibliography on the origin and descent of domestic animals. Fieldiana Anthrop. Chicago, 54 (1), 1900.
4. Aoki, T., Hammerling, U., de Harven, E., Boyse, E.A., and Old, L.J. 1969. Antigenic structure of cell surfaces. An immunoferritin study of the occurrence and topography of H-2, Θ and TL alloantigens on mouse cells. J. Exp. Med. 130, 979.
5. Bach, F.H., and Hirschhorn, K. 1964. Lymphocyte interaction: potential histocompatibility test in vitro. Science 143, 813.
6. Bach, F.H., Bach, M.L., and Klein, J. 1972. Genetic and immunological complexity of major histocompatibility regions. Science 176, 1024.
7. Bain, B., Vos, M.R., and Lowenstein, L. 1964. Development of large immature mononuclear cells in mixed leukocyte cultures. Blood, 23, 108.
8. Balner, H., and Dersjant, H. 1965. Iso-antibodies against leukocytes as a tool to study histocompatibility in monkeys. *In*: Balner, H., Cleton, F.J., and Eernisse, J.G. (eds.). Histocompatibility Testing 1965, p. 103. Munksgaard, Copenhagen.

9. Balner, H., Leeuwen, A. van, Dersjant, H., and Rood, J.J. van 1967. Chimpanzee iso-antisera in relation to human leukocyte antigens. *In*: Curtoni, E.S., Mattiuz, P.L. and Tosi, R.M. (eds.) Histocompatibility Testing 1967, p. 257. Munksgaard, Copenhagen.
10. Balner, H., D'Amaro, J., Toth, E.K., Dersjant, H., and Vreeswijk, W. van 1973. The histocompatibility complex of rhesus monkeys. Relation between RhL-A and the main locus controlling reactivity in mixed lymphocyte cultures. Transplantation Proceedings 5, 323.
11. Balner, H., Dorf, M.E., Groot, M.L. de, and Benacerraf, B. 1973. Histocompatibility complex of rhesus monkeys. III Evidence for a major MLC locus and histocompatibility linked Ir genes. Transplantation Proceedings (In press).
12. Balner, H., D'Amaro, J., and Visser, T.P. 1973. Tissue typing of chimpanzees. Evidence for 2 allelic series of leucocyte antigens. Transplantation Proceedings (In press).
12a. Balner, H., Vreeswijk, W. van, Groot, M.L. de, and D'Amaro, J. The major histocompatibility complex of rhesus monkeys. IV Serological identification of several new antigens of both series of RhL-A, 1973. Transplantation Proceedings in press.
13. Beleil, O.M., Mickey, M.R., and Terasaki, P.I. 1972. Comparison of male and female kidney transplant survival rates. Transplantation 13, 493.
14. Belzer, F.O. 1971. Rôle of preservation in clinical renal transplantation. Transplantation Proceedings 3, 268.
15. Bodmer, J.G., and Bodmer, W.F. 1970. Studies on African Pygmies IV. A comparative study of the HL-A polymorphism in the Batinga Pygmies and other African and Caucasian populations. Amer. J. hum. Genet. 22, 396.
16. Bodmer, W.F., Tripp, M., and Bodmer, J.G. 1967. Application of fluorochromatic cytotoxicity assay to human leukocyte typing. *In*: Curtoni, E.S., Mattiuz, P.L., and Tosi, R.M. (eds.) Histocompatibility Testing 1967, p. 345. Munksgaard, Copenhagen.
17. Bodmer, W.F., Cann, H., and Piazza, A. 1973. Differential genetic variability among polymorphisms as an indicator of natural selection. *In*: Dausset, J. and Colombani, J. (eds). Histocompatibility Testing 1972, p. 753. Munksgaard, Copenhagen.
18. Brent, L., and Medawar, P.B. 1963. Tissue transplantation. A new approach to the "typing" problem. Br. M.J. 2, 269.
19. Bewerton, D.A., Caffrey, M., Hart, F.D., James, D.C.O., Nichols, A. and Sturrock, R.D. 1973. Ankylosing spondylitis and HL-A 27. Lancet i, 904.
20. Bruning, J.W., Douglas, R., Scholtus, M. and Rood, J.J. van, 1972. Automatic reading and recording of the microlymphocytotoxicity test. Tissue Antigens 2, 473.
21. Cavalli-Sforza, L.L., and Bodmer, W.F. 1971. The genetics of human populations. Chapter 11, Freeman and Company, San Francisco.
22. Ceppellini, R., Curtoni, E.S., Mattiuz, P.L., Miggiano, V., Scudeller, G. and Serra, A. 1967. Genetics of leukocyte antigens. A family study of segregation and linkage. *In*: Curtoni, E.S., Mattiuz, P.L. and Tosi, R.M. (eds.) Histocompatibility Testing 1967, p. 149. Munksgaard, Copenhagen.
23. Chai, C.K. and Chaing, M.S.M. 1963. A method of estimating the number of histocompatibility loci in a sib mating mouse population. Genetics 48, 153.
24. Cleton, F.J. 1965. Iso-antibodies in dogs. *In*: Balner, H., Cleton, F.J. and Eernisse, J.G. (eds.) Histocompatibility Testing 1965, p. 263. Munksgaard, Copenhagen.
25. Cleton, F.J., Es, G. van, Ponsen, R., and Rood, J.J. van, 1967. Leucocyte antigens in the dog. *In*: Curtoni, E.S., Mattiuz, P.L. and Tosi, R.M. (eds.) Histocompatibility Testing 1969, p. 277. Munksgaard, Copenhagen.
26. Cochrum, K.C., Perkins, H.A., Payne, R.O., Kountz, S.L. and Belzer, F.O. 1973. The

correlation of MLC with graft survival. Transplantation Proceedings 5, 391.
27. D'Amaro, J. 1970. The microcomplement fixation test. I. A solution of the technical problems of HL-A typing? Vox. Sang. 18, 334.
28. Dausset, J. and Hors, J. 1973. Statistics of 416 consecutive kidney transplants in the France Transplant Organization. Transplantation Proceedings 5, 223.
29. Does, J.A. van der, Rood, J.J. van, Walker, W. and Epstein, R.B. 1973. Consequent intrafamilial immunization for DL-A haplotyping in canines. J. Exp. Med. 137, 494.
30. Dorf, M.E., Toth, E.K., and Balner, H. 1972. Cross-reactions of HL-A antibodies. V. Relationship between the human HL-A 11 and chimpanzee ChW-11 specificities. Tissue Antigens 2, 461.
31. Einstein, A.B., Mann, D.L., Gordon, H.G., Trapani, R. and Fahey, J.L. 1971. Heterologous antisera against specific HL-A antigens. Transplantation 12, 299.
32. Eijsvoogel, V.P., Rood, J.J. van, du Toit, E.D. and Schellekens, P.Th.A. 1972. Position of a locus determining mixed lymphocyte reaction, distinct from the known HL-A loci. Eur. J. Immunol. 2, 413.
33. Eijsvoogel, V.P., du Bois, M.J.G.J., Melief, C.J.M., Groot-Kooy, M.L., Koning, C. de, Rood, J.J. van, Leeuwen, A. van, du Toit, E. and Schellekens, P.Th.A. 1973. Position of the locus determining mixed lymphocyte reaction (MLR). Distinct from the known HL-A loci and its relation to cellmediated lympholysis (CML). *In*: J. Dausset and J. Colombani (eds.). Histocompatibility Testing 1972, p. 501. Munksgaard, Copenhagen.
34. Epstein, R.B., Storb, R., Ragde, H. and Thomas, E.D. 1968. Cytotoxic antisera for bone marrow grafting in littermate dogs. Transplantation 6, 45.
35. Falchuck, Z.M., Rogentine, G.N. and Strober, W.J. 1972. Predominance of histocompatibility antigen HL-A 8 in patients with gluten-sensitive enteropathy. J. Clin. Invest. 51, 1602.
36. Falconer, D.S. 1964. Introduction to quantitative genetics. Oliver and Boyd, Edinburgh.
37. Ferrebee, J.W., Cannon, F.D., Mollen, N. and St. John, D. 1970. Beagles for studies of histocompatibility and organ transplantation. Transplantation 9, 68.
38. Fisher, R.A. 1930. The genetical theory of natural selection. (2nd. ed., 1958, New York, Dover).
39. Ford, E.B. 1965. Genetic polymorphism. Faber and Faber, London.
40. Gabb, B.W., Piazza, A., D'Amaro, J. and Balner, H. 1972. Genetics of RhL-A system of Rhesus Monkeys. Transplantation Proceedings 4, 11.
41. Gatti, R. and Good, R.A. 1971. Malignancy in immunodeficiency diseases. Cancer 28, 89.
42. Gershowitz, H. and Neel, V.J. 1970. *In*: Aminoff, D. (ed.). Blood and tissue antigens, p. 33. Academic Press Inc., New York.
43. Gorer, P.A. 1938. The antigenic basis of tumor transplantation. J. Path. Bact. 47, 231.
44. Graff, R.J., Silvers, W.K., Billingham, R.E., Hildemann, W.H., and Snell, G.D. 1966. The cumulative effect of histocompatibility antigens. Transplantation 4, 605.
45. Grumet, F.C., Couhell, A., Bodmer, J.C., Bodmer, W.F. and McDevitt, H.O. 1971. Histocompatibility antigens associated with systemic lupus erythematodes. New Engl. J. Med. 285, 193.
46. Günter, E., Rüde, E. and Stärk, O. 1972. Antibody response in rats to the synthetic polypeptide (T,G)-A-L genetically linked to the major histocompatibility system. Eur. J. Immunol. 2, 151.
47. Hamburger, J., Crosnier, J., Descamps, B. and Rowinska, D. 1971. The value of

present methods used for the selection of organ donors. Transplantation Proceedings 3, 260.
48. Harris, H. 1969. Enzyme and protein polymorphism in human populations, Brit. Med. Bull 25, 5.
49. Harris, H. 1971. Polymorphism and protein evolution. The neutral mutation-random drift hypothesis. J. Med. Gen. 8, 444.
50. Harris, R. and Zervas, J.D. 1969. Reticulocyte HL-A antigens. Nature 22, 1062.
51. Hildeman, W.H. 1970. Immunogenetics. Chapter 1, Holden Day Inc., San Francisco.
52. Hirata, A.A. and Terasaki, P.I. 1970. Cross reactions between streptococcal M proteins and human transplantation antigens. Science 168, 1095.
53. Hooff, J.P. van, Steen, G.J. van der, Schippers. H.M.A. and Rood, J.J. van, 1972. Efficacy of HL-A matching in Eurotransplant. Lancet i, 1385.
54. Jensen, C.O. 1903. Experimentelle Untersuchungen über Krebs bei Mäusen. Centralbl. J. Bakt etc. I. Abt. Originale Bd. XXXIV. 2, 122.
55. Kimura, M. 1968. Evolutionary rate at the molecular level. Nature 217, 624.
56. Kissmeyer-Nielsen, F., Kjerbye, K.E. 1967. Lymphocytotoxic microtechnique purification of lymphocytes by flotation. *In*: Curtoni, E.S., Mattiuz, P.L. and Tosi, R.M. (eds.) Histocompatibility 1967, p. 381. Munksgaard, Copenhagen.
57. Kissmeyer-Nielsen, F. et al. 1971. Scandia transplant. Preliminary Report of a kidney exchange program. Transplantation Proceedings 3, 1019.
58. Koldovsky, P. 1969. Tumor Specific Transplantation Antigens. Springer Verlag, Berlin.
59. Kourilsky, F.M., Silvestre, D., Levy, J.P., Dausset, J., Nicolai, M.G. and Senil, A. 1971. Immunoferritin study of the distribution of HL-A antigens on human blood cells, J. Immunol. 106, 545.
60. Kren, V., Bílá, V., Krsiahová, M., and Krenova, D. 1972. Genetic determination of polydactyly-luxate and its association with an H antigen. Anim. Blood Grps. Biochem. Genet. 3, 84.
61. Krsiahová, M., Kren, V. and Stark, O. 1972. An attempt to isolate histocompatibility antigens associated with coat colourgenes in rats. Anim. Blood Grps. Biochem. Genet. 3, 86.
61a. Lance, E.M., Boyse, E.A., Cooper, S. and Carswell, E.A. 1971. Rejection of Skin allografts by irradiation chimeras. Evidence for skin specific transplantation barrier. Transplantation Proceedings 3, 864.
62. Landsteiner, K.D. 1900 Zur kenntnis der Anti-Germantativen Lytischen und agglutinierenden Wirkungen der Blutserum und der Lymphe. Zentralbl. Bakt, etc. 27, 357.
63. Landsteiner, K.D. 1931. Individual differences in human blood. Science 73, 403.
64. Leeuwen, A. van, and Rood, J.J. van, 1973. Typing for MLC (LD) II. The selection of non stimulator cells by MLC inhibitions tests using SD identical simulator cells (MICIS) and fluorescent antibody studies. Transplantation Proceedings (In press).
65. Levine, B.B., Ojeda, A. and Benacerraf, B. 1963. Studies on artificial antigens III. The genetic control of the immune response to hapten poly L-Lysine conjugates in guinea pig. J. Exp. Med. 118, 953.
66. Levine, B.B., Stember, R.H. and Fotino, M. 1972. Ragweed Hay Fever: Genetic control and linkage to HL-A haplotypes. Science 178, 1201.
67. Lewontin, R.C. and Hubby, J.L. 1966. A molecular approach to the study of genetic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of Drosophila pseudo obscura. Genetics 54, 595.
68. Li, C.C. 1961. Human Genetics, McGraw Hill Book Inc., New York.

68a. Lilly, F. 1968. The effect of histocompatibility-2 type on response to the Friend leukemia virus in mice. J. Exp. Med. 127, 465.
69. Little, C.C. and Tyzzer, E.E. 1916. Further experimental studies on the inheritance of susceptibility to a transplantable tumour carcinoma (J.W.A.) of the Japanese walking mouse. Med. Research, 33, 393.
70. Matsukura, M., Mery, A.M., Amiel, J.L. and Mathé, G. 1963. Investigations on test of histocompatibility for allogenic grafts II. Study on rabbits. Transplantation, 1, 61.
71. Mattiuz, P.L., Ihde, D., Piazza, A., Ceppellini, K. and Bodmer, W.F. 1970. New Approaches to the population genetic and segregation analysis of the HL-A system. *In*: Terasaki, P.I. (ed.) Histocompatibility Testing 1970, p. 193. Munksgaard, Copenhagen.
72. McDevitt, H.O. and Benacerraf, B. 1969. Genetic control of specific immune responses. Adv. of Immunology 11, 31.
73. McKay, J.K. and Morris, P.J. 1972. Association of auto immune active chronic hepatitis with HL-A-1,8. Lancet ii, 793.
74. McKhann, C.F. 1969. Primary malignancy in patients undergoing immunosuppression of renal transplantation. Transplantation 8, 209.
75. Medewar, P.B. 1857. The immunology of transplantation. Harvey Lectures, Series 52, 144.
76. Mempel, W., Grosse-Wilde, H., Albert, E. and Thierfelder, S. 1973. Atypical MLC reactions in HL-A typed related and unrelated pairs. Transplantation Proceedings 5, 401.
77. Miller, J.F.A.P., Grant, G.A. and Roe, F.J.C. Effects of thymectomy on the induction of skin tumours by 3,4-Benzopyrene. Nature 199, 920.
78. Mittal, K.K., Terasaki, P.I., Springer, G.F., Desai, P.R., McIntire, F.C. and Hirata, A.A. 1973. Inhibition of anti HL-A alloantisera by glycoproteins, polysaccharides and lipopolysaccharides from diverse sources. Transplantation Proceedings 5, 499.
79. Morris, P.J. and Forbes, F. 1971. Further studies of HL-A. Transplantation Proceedings 3, 1275.
80. Morton, J.A., Pickles, M.M. and Sutton, L. 1969. The correlation of the Bga blood-group with the HL-A 7 leucocyte group. Demonstration of antigenic sites on red cells and leucocytes Vox. Sang. 17, 536.
81. Murphy, E.D. 1966. *In*: Green, E.L. (ed.). Biology of the Laboratory Mouse, 2nd. edition, p. 521. McGraw Hill, Inc., New York.
82. Naito, S., Namerow, N., Mickey, M.R. and Terasaki, P.I. 1972. Multiple Sclerosis, association with HL-A 3. Tissue Antigens 2, 1.
83. Novinsky, M. 1876. Zur Frage über die Impfung der krebsigen Geschwülste. Zentralbl. f. Med. Wissensch. 14, 790.
84. Oldstone, McDevitt and Mitchill, 1972. *In*: McDevitt, H.O. and Landry, M. (eds.). Genetic control of immune responsiveness. Relationship to disease susceptibility. p. 300. Academic Press, New York.
85. Opelz, G., Sengar, P.P.S., Mickey, M.K. and Terasaki, P.I. 1973. Effect of blood transfusions on subsequent kidney transplants. Transplantation Proceedings 5, 253.
86. Patel, R., Mickey, M.R., and Terasaki, P.I. 1969. Leucocyte antigens and disease I. Association between HL-A 2 and chronic glomerulonephritis. Br. Med. J. ii, 424.
87. Palm, J. 1964. Serological detection of histocompatibility antigens in two strains of rats. Transplantation 2, 603.
88. Payne, R. and Rolfs, M. 1958. Fetometernal leucocyte incompatibility. J. Clin. Invest. 37, 1756.
89. Piazza, A., Belvedere, M.C., Bernoco, D., Corrighi, C., Contu, L., Curtoni, E.S., Mat-

tiuz, P.L., Mayr, W., Richiardi, P., Scudeller, G. and Ceppellini, R. 1973. HL-A variation in four Sardinian Villages under differential selective pressure by malaria. *In*: Dausset, J. and Colombani, J. (eds.). Histocompatibility testing 1972, p. 73. Munksgaard, Copenhagen.

90. Pirskanen, R., Tiillikainen, A. and Hohkaner, E. 1972. HL-A antigens in myasthenia gravis. Ann. Clin. Res. 4, 304.
91. Ramseier, H. 1866. *In*: Largadèr, F. (ed.) Histocompatibilität und ihre Testung. Organtransplantation, p. 85. Thieme Verlag, Stuttgart.
91a. Rehfeld, C.E. 1970. Definition of Relationship in a closed Beagle Colony, Am. J. Vet. Res, 31, 723.
92. Rood, J.J. van, Eernisse, J.G. and Leeuwen, A. van, 1958. Leucocyte antibodies in sera from pregnant women. Nature 181, 1735. London.
93. Rood, J.J. van, 1962. Thesis. University of Leyden, The Netherlands.
94. Rood, J.J. van, Leeuwen, A. van, Graaf, S. de, and Mowbray, J. 1971. Relationship between kidney graft prognosis and HL-A typing using a micro-ansorption technique. Transplantation Proceeding 3, 1051.
95. Rood, J.J. van, Koch, C.T., Hoof, J.P. van, Leeuwen, A. van, Tweel, J.G. van den, Frederiks, E., Schippers, H.M.A., Hendriks, G. and Steen, G.J. van der, 1973. Graft survival in unrelated donor-recipient pairs. Matched for MLC and HL-A. Transplantation Proceedings 5, 409.
96. Rood, J.J. van, 1973. LD-SD interaction in vivo and the allograft reaction. Transplantation Proceedings (In press).
97. Rubinstein, P., Morgado, F., Blumenstock, D.A. and Ferrebee, J.W. 1968. Isohemaglutinins and histocompatibility in the dog. Transplantation 6, 961.
98. Schlosstein, L., Terasaki, P.I., Bluestone, R., Pearson, C.M. 1973. High association of an HL-A antigen, W 27, with ankylosing spondylitis. New Engl. J. Med. 288, 704.
99. Shoskiewicz, M., Chase, C., Winn, H.J. and Russell, P.S. 1973. Kidney transplants between mice and graded immunogenetic diversity. Transplantation Proceedings 5, 721.
100. Snell, G.D. 1948. Methods for the study of histocompatibility genes. J. Genet. 49, 87.
101. Snell, G.D. and Stimpling, J.H. 1966. *In*: Green, E.L. (ed.). Genetics of tissue transplantation. Biology of the laboratory mouse, 2nd. edition. p. 457. McGraw-Hill Book Company, New York.
102. Stimpling, J.H., Boyse, E.A. and Mitshell, K. 1964. Preparation of isoantisera in laboratory mice. Meth. Med. Res. 10, 18.
103. Stokes, P.L., Asquith, P., Holmes, G.K.T. and Mackintosh, P. and Cook, W.T. 1972. Histocompatibility antigens associated with adult coeliac disease. Lancet ii, 162.
104. Svejgaard, A. and Kissmeyer-Nielsen, F. 1968. Cross-reactive human HL-A iso-antibodies. Nature 219, 868. London.
105. Terasaki, P.I. and McClelland, J.D. 1964. Microdroplet assay of human serum cytotoxins. Nature 219, 998. London.
106. Tissot, R.G. and Cohen, C. 1972. Histocompatibility in the rabbit. Tissue Antigens 2, 267.
107. Viza, D., Sugar, J.A. and Binns, R.M. 1970. Lymphocyte stimulation in Pigs. Evidence for the existence of a single major histocompatibility Locus PL-A. Nature 227, 949.
108. Vogel, F., Pettenhofer, H.J. and Helmbold, W. 1960. Uber die Population-genetik der ABO Blutgruppen 2. Mitteilung Genhäufigkeit und epidemische Erkrankungen. Acta Genet. Statist. Med. 10, 267.

109. Weck, A.L. de, Polak, L., Sato, W. and Frey, J.K. 1971. Determination of histocompatibility antigens by leucocyte typing in outbred guinea pigs and effect of matching on skin graft survival. Transplantation Proceedings 3, 192.
110. Williamson, M.H. 1958. Selection, controlling factors and polymorphism. The American Naturalist 42, 329.
111. Wilson, R.E., Henry, C. and Merrill, J.P. 1963. Model system for determining histocompatibility in man. J. Clin. Invest. 42, 1497.
112. Yunis, E.J., Ward, F.E. and Amos, D.B. 1970. Observations of the CNAP Phenomenon. *In*: Terasaki, P.I. (ed.). Histocompatibility Testing 1970. p. 351. Munksgaard, Copenhagen.

CHAPTER 2

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THE PRODUCTION AND EVALUATION OF DOG ALLO-LYMPHOCYTOTOXINS FOR DONOR SELECTION IN TRANSPLANTATION EXPERIMENTS

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SUMMARY

The production of tissue-typing reagents for dog leucocytes by allografting procedures is described. Skin and small bowel grafts were efficient in producing informative sera. A negative correlation was found between the duration of graft survival and the subsequent development of cytotoxic antibodies. It was shown that these reagents were useful in the selection of littermate donor-recipient pairs for transplantation experiments. Evidence presented from family testing and tissue transplantation supports the existence of a major (*DL-A*) histocompatibility locus in dogs.

The dog is an animal commonly used for transplantation research. Relatively little is known about canine histocompatibility (*4, 5*). In our laboratories, the influence of histocompatibility testing on allograft survival and the histological signs of rejection are being studied. Westbroek et al. (*14*) reported the results of canine lymphocyte typing on the survival of heterotopic, small intestine allografts. The survival periods of orthotopic heart allografts in identical and non-identical beagle littermates were published by Bos et al. (*3*).

Experiments on the influence of immunosuppressive therapy on graft survival in matched and mismatched donor-recipient pairs have been started in our laboratories. To prove the existence of organ-specific antigens in the dog is another aim of our studies. The purpose of this communication is to report how informative tissue-typing reagents were obtained and evaluated in studies of beagle families.

METHODS

The microcytotoxicity test of Kissmeyer-Nielsen, modified by van Rood et al. (*12*), was used to test dog lymphocytes. Skin allografts were done following the technique described by Balner (*1*). Small bowel allografts were done by the technique described by Lillehei (*8*) and Grenier et al. (*6*).

Three immunosuppressive regimes were used. (1) Some animals were treated with Imuran. Doses were given in milligrams per kilogram of body weight, as follows: 6 mg on postoperative days 0–5, 3 mg on postoperative days 6–13, 2 mg on postoperative day 13, and 1 mg/day until rejection of the graft occurred. (2) Horse anti-dog lymphocyte sera (HADLS) were given s.c., with 50 mg of Ig/kg of body weight as the unit dose. On preoperative day −2, two doses were given. In the first 3 postoperative weeks, 3 doses/week were given. In the 4th and 5th postoperative weeks, 2 doses/week were administered, and in the 6th postoperative week, 1 dose. (3) Another group of dogs was treated with Imuran and HADLS, following the schedules described above.

The sera of dogs that rejected skin or small intestine allografts were harvested 8–10 days after rejection and were tested for the presence of allolymphocytotoxins against the cells of 40 random beagles. All animals were boosted by the s.c. injection of whole blood at five different sites. A serum sample was taken 5–8 days after boosting. In selected cases, boosting was repeated at 1 month.

Beagles were obtained from the Centraal Proefdierenbedrijf T.N.O. (Zeist, The Netherlands; Dr. J. van Vliet) and our own colony. The family studies were done in those colonies.

RESULTS

Production of typing sera. Forty-four sera harvested after rejection of skin or small bowel homografts were tested against the lymphocytes of a standard panel of 40 random beagles with the microcytotoxicity test (Table 1). Sera were considered pattern 1 if they gave negative or doubtful positive results with the cells of the test dogs. Sera were pattern 3 if they gave positive results with more than 75% of the dogs tested. Sera with pattern 2 were the so called "informative group" with positive results in less than 75% of the dogs.

Boosting 10 dogs with serum of pattern 1 did not yield "informative sera." Their sera remained of pattern 1 or developed a pattern 3. It took 6 months or more before 7 dogs with sera pattern 3 developed a lower frequency of positive results with the 40 cell samples.

Informative sera from dogs remained useful when harvested in the first 2½ months after rejection, without boosting. Boosting at that time or later produced, in 2 of 10 dogs tested, sera with a higher frequency of positive results with the cells of the standard group.

Skin grafts (nonvascularized) were compared to small bowel grafts (vascularized) for their efficacy in producing useful typing sera (Table 1). Skin grafting seemed to be a better method of producing typing sera, although this difference in efficacy was not statistically significant.

By comparing long, intermediate, and short survivals in skin and small bowel allografts (Table 2), a statistically significant ($P < 0.001$) correlation between serum patterns and survival periods (r, 0.64) was observed (*7*). Survivors that maintained the grafts for prolonged periods did not produce antibodies in most cases (pattern 1), while short graft survival was most commonly associated with antibody production of patterns 2 and 3.

Animals receiving allografts from more than one donor and animals used to test HADLS in vivo in skin allografts are not included in Table 2.

Studies of cytotoxicity results in beagle families. Twenty sera with an informative pattern, obtained in Rotterdam, and 38 sera raised by Cleton (*4*) were used to tissue type beagle families. At the time of the study, no "operationally" monospecific antisera (*13*) recognizing defined dog leucocyte antigens were known.

Most of our antisera gave positive results with more than 50% of the beagle colony. Nevertheless, we were able to divide littermate beagle pairs into three different histocompatibility groups, if we assumed the existence of one major dog histocompatibility locus (*DL-A*) and that our sera recognized mixtures of antigens of this locus.

Table 3 shows an example of the haplotyping of a family by a panel of 58 sera. Although the

TABLE 1. Serum patterns 8–10 days after rejection of different allografts

Allograft	Sera with pattern			
	1	2	3	Total
Small intestine	10	5	3	18
Skin from one donor	5	9	2	16
Skin from two donors or more	0	4	6	10
Total	15	18	11	44

TABLE 2. Survival periods of allografts compared with serum patterns after rejection[a]

Survival period[b]	Score	(Pattern): 1	2	3	Total sera
		(Score): 1	2	3	
Short	3	1	6	4	11
Intermediate	2	2	2	—	4
Long	1	10	4	—	14
Total		13	12	4	29

[a] Correlation of graft survival to serum patterns significant at $P < 0.001$.

[b] Short survival—skin grafts and small intestine grafts rejected within 10 days after transplantation; small intestine grafts rejected within 20 days after transplantation when treated with antilymphocyte serum (ALS), Imuran, or both. Intermediate survival—skin grafts rejected between 11 and 16 days; small intestine grafts rejected between 10 and 20 days; small intestine grafts treated with ALS, Imuran, or both, rejected between 20 and 40 days. Long survival—skin grafts rejected in 16–21 days; small intestine grafts rejected after day 20; small intestine grafts treated with ALS, Imuran, or both, rejected after day 40.

TABLE 3. Analysis of cytotoxicity results in beagle family 36[a]

Family member	Results with sera[b]						Expected haplotype of father (A or B)	Results with sera[c]					Expected haplotypes of mother (C or D)	Expected haplotype of litter	Results with sera[d]			
	11	12	24	27	34	38		59	49	50	55	58			16	17	23	43
Father	++	++	++	+′	++′	++		−	−	−	−	−			+++	+++′	+++	++′
Mother	−	−	−	(+)	−	−		++++	+++′	++′	++′	+			++	++′	++	++′
Littermates																		
1 ♂	+++	++′	++	++	+++	+++	A	++′	+++	+++′	++′	++	D	AD	−	+++	+++	−
2 ♂	++′	++	+′	+	+++	++′	A	+′	++′	+++′	++′	++′	D	AD	−	+++	+++	−
3 ♂	+++	++′	+′	++′	+++	++′	A	++′	++	+++	+++	++	D	AD	−	+++	+++	−
4 ♂	−	−	−	−	+	+′	B	++′	++++	++′	+++′	+	D	BD	++′	++	−	−
5 ♀	++	−	++	−	−	−	B	−	−	−	−	−	C	BC	+++	−	++′	+++′
6 ♀	+++	++	++	+′	++++	+++	A	−	−	−	−	−	C	AC	+++	+++	++′	++′
7 ♀	+++	++′	++′	++	+++	+++	A	(+)	−	−	−	−	C	AC	++	+++′	++′	+′
8 ♀	++′	++′	+′	+′	+++	++	A	++	(+)	+′	++	(+)	D	AD	−	+++	++	−
Haplotypes recognized	A	A	A	A	A	A		D	D	D	D	D			BC	AD	AC	C/BC ?

[a] Only those panel sera are shown which gave clear positive and negative results among the litter.
[b] Positive reactions with father; negative with mother.
[c] Negative reactions with father; positive with mother.
[d] Positive reactions with both parents.

actual antigens of the DL-A haplotypes of the parents were not clearly recognized, we were able to locate the parental haplotypes A and B for the father and C and D for the mother, in the litter. In the group of sera that reacted positively with the father's lymphocytes and negatively with the mother's, two different types of sera could be present—one type following haplotype A and one following haplotype B in the litter. The groups of sera negative with the father's lymphocytes and positive with the mother's can show also two different patterns of results in the litter, one indicating haplotype C and one, D. In the family shown (Table 3), only haplotypes A and D are recognized. In other families, we sometimes found sera for all four haplotypes.

In the depicted family, siblings 1, 2, 3, and 8 are examples of *DL-A*-identical littermates, 3 and 4 differ in one DL-A haplotype, and 5 and 8 have two DL-A haplotype differences. With the described panel of test sera, we were able to recognize 58% of the DL-A haplotypes in 50 beagle families. With a new panel, from which we removed 18 sera of the first panel and to which we added 19 more recently produced informative sera, we succeeded in recognizing 75% of the DL-A haplotypes in 40 beagle families from the same parents.

Evidence for the existence of DL-A. Table 4 shows the segregation analysis of parental chromosomes as described by Mattiuz et al. (*9*). We studied only the beagle families in which the paternal and/or maternal haplotypes showed a clear-cut segregation in the litter.

In the family shown in Table 3, the paternal segregation ratio is 6:2 (A:B), the maternal segregation ratio, 5:3 (D:C). The low χ^2 values show an excellent fit with the hypothesis that our antisera recognize an antigenic system which segregates in the F_1, following the laws of Mendel for a hereditary trait governed by one chromosomal locus. Figure 1 shows the graft survival of different organs exchanged between beagle littermates. Each bar is the medium graft survival time of six allografts or more. The survival periods correlate very well with the haplotype differences between donor and recipient.

These results indicate that the antigenic system recognized by our antisera is governed by *one* chromosomal locus which has overriding importance for allograft survival. In analogy to the *HL-A* system for human leucocytes, this locus has been called *DL-A*.

DISCUSSION

Our results clearly show that the production of typing sera is rather easy in transplantation laboratories. Postpartum sera were, in our experience, less successful. Only one informative serum was obtained out of eight bitches tested.

The i.v. or s.c. injections of cells to raise dog alloantisera have the disadvantage that long immunization schedules are necessary. It is reported in the literature that after long immunization schedules antibodies lose their specificity

TABLE 4. Paternal and maternal DL-A chromosomes segregating in litters of different sizes[a]

Litter size (s)	Segregation ratio of haplotypes[b]	No. of litters with paternal segregation	No. of litters segregating		χ^2	No. of litters with maternal segregation	No. of litters segregating		χ^2
			Expected	Observed			Expected	Observed	
4	3:1	8	4.57	5	0.09	8	4.57	5	0.09
	2:2		3.43	3	(1 DF)		3.43	3	(1 DF)
5	4:1	13	4.33	5	0.15	12	4	2	1.50
	3:2		8.67	8	(1 DF)		8	10	(1 DF)
6	5:1	22	4.25	4	0.79	23	4.45	7	1.89
	4:2		10.65	9	(2 DF)		11.13	9	(2 DF)
	3:3		7.10	9			7.42	7	
7	6:1	18	2	0	2.27	17	1.89	2	0.13
	5:2		6	7	(2 DF)		5.67	5	(2 DF)
	4:3		10	11			9.33	10	

[a] Analysis as described by Mattiuz et al. (*9*).

[b] The chances of the different segregation patterns follow the coefficients of the formula $(\frac{1}{2} + \frac{1}{2})^s$. The segregation pattern s:0 is not recognized for litter size s.

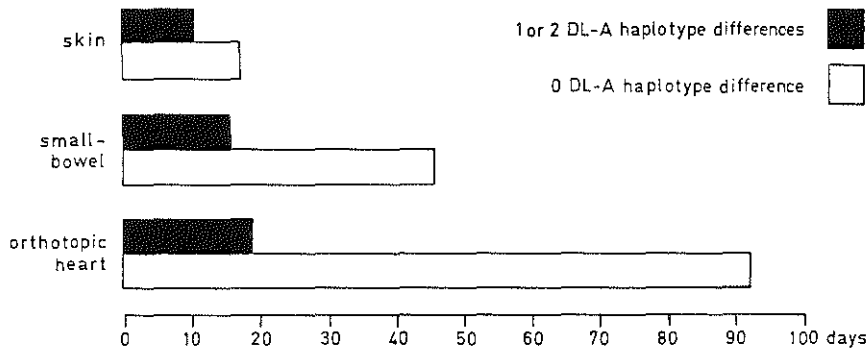

FIGURE 1. Mean survival times of different allografts in identical and nonidentical beagle littermates (without immunosuppressive therapy).

and gain avidity. Therefore, we consider allografting of solid organs the best way to produce dog alloantisera (*2*).

It is possible that, instead of s.c. injections, a second allograft from the same donor would be a better way of boosting. This possibility will be investigated in further experiments. Until more is known about the major dog histocompatibility locus, the described methods and the produced sera will enable us to tissue type littermate beagles with sufficient accuracy to be able to select identical and nonidentical littermates.

The existence of a major dog histocompatibility locus is very probable, as shown by the results of our studies, and is in accordance with the results reported by Epstein et al. (*5*) and Rapaport et al. (*10*).

We follow several approaches in trying to define dog leucocyte antigens. First, antisera with common reactivity are identified. For this the computer has been helpful (*11*). Second, absorption and elution studies are done for refinement of antigenic recognition of the antisera. Finally, planned transplantations with known DL-A antigen differences are done. We expect to be able to report in the near future on DL-A antigens found by these approaches. The knowledge of defined DL-A antigens will also facilitate the typing of random dogs.

It is possible that dog leucocyte antigens are different in various parts of the world and in various breeds, because of selective breeding and/or genetic drift. Therefore, it would be most profitable to exchange typing reagents between dog laboratories.

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REFERENCES

1. Balner, H. 1969. Transplantation *8:* 206.
2. Balner, H.; Dersjant, H. 1965. p. 103. *In* H. Balner, F. J. Cleton, and J. G. Eernisse (eds.). *Histocompatibility testing 1965.* Munksgaard, Copenhagen.
3. Bos, E.; Meeter, K.; Israel, D. E.; Stibbe, J.; van Rood, J. J. 1970. Europ. Surg. Res. *2:* 93.
4. Cleton, F. J. 1965. p. 263. *In* H. Balner, F. J. Cleton, and J. G. Eernisse (eds.). *Histocompatibility testing 1965.* Munksgaard, Copenhagen.
5. Epstein, R. B.; Storb, B.; Ragde, H.; Thomas, E. D. 1968. Transplantation *6:* 45.
6. Grenier, J. F.; Santizo-Lepe, G.; Feltgen, F.; Beiger, C.; Adriansen, D.; Weiss, A. G. 1966. *Premier congrès de la Société Européen de Chirurgie Expérimentale,* Lyon.
7. de Jonge, H. (ed.). p. 643. 1963. *Inleiding tot de medische statistiek II.* Nederlands Instituut voor Preventieve Geneeskunde, Leiden.
8. Lillehei, R. C. 1962. Surg. Clin. N. Amer. *42:* 1191.
9. Mattiuz, P. L.; Ihde, D.; Piazza, A.; Ceppellini, R.; Bodmer, W. F. 1970. p. 193. *In* P. I. Terasaki (ed.). *Histocompatibility testing 1970.* Munksgaard, Copenhagen.
10. Rapaport, F. T.; Hanaoka, T.; Shimada, T.; Cannon, F. D.; Ferrebee, J. W. 1970. J. Exp. Med. *131:* 881.
11. van Rood, J. J. 1962. Ph.D. Thesis. University of Leiden, Leiden, The Netherlands.

12. van Rood, J. J.; van Leeuwen, A.; Zweerus, R. 1970. p. 93. *In* P. I. Terasaki (ed.). *Histocompatibility testing 1970*. Munksgaard, Copenhagen.
13. Walford, R. L.; Shandrom, E.; Troup, C. M.; Zeller, E.; Ackerman, B. 1967. p. 221. *In* E. S. Curtoni, P. L. Mattiuz, and R. M. Tosi (eds.). *Histocompatibility testing 1967*. Munksgaard, Copenhagen.
14. Westbroek, D. L.; Rothengatter, C.; van Rood. J. J. 1970. Europ. Surg. Res. *2:* 155.

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CHAPTER 3

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POLYMORPHISM OF THE DL-A SYSTEM

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SUMMARY

The major histocompatibility system of the dog (DL-A) was studied in more detail by immunogenetical methods. Suggestive evidence for the existence of two closely linked *DL-A* (sub)loci was found. The definition of three alleles at the first sublocus was clear for both mongrel and beagle populations. Three additional specificities appear to recognize antigens of a second sublocus, at least in the beagle colony. Differences in the frequency of the described specificities were shown between the mongrel and the beagle populations.

The existence of a major histocompatibility system, located on one or several linked loci of one chromosome pair is well established in man (HL-A) (*1*), mouse (H-2) (*16*), rat (Ag-B) (*11*), rhesus monkey (RhL-A) (*4*), chicken (B) (*9*), and pig (PL-A) (*22*). A similar genetic system in dogs (DL-A) has been anticipated (*12*). Evidence for the existence of this system has been obtained by serological and graft survival studies (*5, 23, 26*) and by mixed lymphocyte culture experiments (*18*).

The purpose of this article is to describe the experiments performed to investigate the DL-A system in more detail.

Immunogenetical methods were applied to the results of cytotoxicity tests performed on the cells of 100 random mongrel dogs and 77 random beagles with 26 selected canine alloantisera. The same dog alloantisera were used to type the cells of 70 beagle and 10 mongrel families. An attempt was made to explain the obtained results by the assumption of the existence of one series or more of multiple codominant alleles in the chromosomal DL-A region.

MATERIAL AND METHODS

Dogs

Mongrel dogs and their families were obtained from local dog handlers and breeders. Beagle families were studied in a colony of Het Centraal Proefdierenbedrijf TNO, Austerlitz, Holland (Dr. J. van Vliet) and in our own colony (Dr. W. van Dijk).

The group of random beagles was composed of animals which came from six different beagle breeders. Beagles which were bought from one breeder were considered random when pedigrees were available showing that the animals were unrelated or that they were born on different days. However, the possibility that some of the beagles included in the random group had a common ancestor could not be completely excluded.

Alloantisera

The production and evaluation of dog alloantisera have been described previously (*23, 24*). Briefly, dog alloantisera were obtained after multiple injections of leucocyte suspensions, after pregnancies, or after the rejection of transplanted solid organs. Our method of choice at the moment is a skin allograft exchanged between dog littermates that share one parental chromosome, carrying the genetic information for DL-A (one DL-A haplotype difference).

Antisera were screened for antibody activity against the cells of at least 40 random beagles or mongrel dogs. Twenty-six antisera from 26 different dogs (4 mongrels and 22 beagles) had promising screening results (clear positive reactions with a population frequency of 0.50 or less) and were used as the panel of test sera.

The random mongrel dogs and beagles were

tested against this panel in duplicate on 2 different days. The beagle families were tested once, while the mongrel families were tested on at least two different occasions.

Cytotoxicity Test

The microcytotoxicity test as described by Kissmeyer-Nielsen and modified by van Rood et al. (*21*) was used. In these studies this test had a reproducibility of more than 85%.

Immunogenetical Methods

Population studies. The reactivity patterns of the antisera in the two dog populations were analyzed with the help of computer programs (*13*). Sera with common reactivity patterns were analyzed as a group (cluster).

In general, only those typing results were used for further analysis that showed a concordant negative or positive cytotoxicity test on two different occasions. Discordant results were regarded as "not done" and were always left out from the analysis except in the data in Tables 3 and 4 when these results were scored as negative.

Results of the cytotoxicity tests of the lymphocytes of the random dogs were compared in 2×2 contingency tests. Two different χ^2 tests, described extensively by Andresen et al. (*2*), were used. The first χ^2 test has been called χ^2 test for independence, because it assumes that the reactivity patterns of the two antisera in question are independent. In this test a χ^2 value >3.8 shows ($P < 0.05$) a positive association between the two antisera when $ad > bc$ or a negative association when $bc > ad$ (a, b, c, and d being, respectively, the $+ +$, $- +$, $+ -$, and $- -$ results in the 2×2 table).

The second χ^2 test has been named χ^2 test for allelism. It assumes that the two antisera in question detect two specificities that are controlled by two different alleles of one genetic system which are in Hardy-Weinberg equilibrium in the population in study.

A χ^2 value >3.8 in this test is not compatible with an allelic relationship of the two specificities in question. The rationale of using two different χ^2 tests is that the χ^2 values of either test often do not reach statistically significant levels in a small sample, especially when the gene frequencies of the studied specificities are low. In such a case the most probable relationship between the two specificities can nevertheless be found, if the two different χ^2 tests support the same of the two alternatives (allelism or independence).

If, by applying these tests, evidence is found for the existence of a system of multiple alleles, no subject in the population should possess more than two specificities of one series of multiple alleles (absence of "triplets"). Gene frequencies of the studied specificities were determined by using the formula, $GF = 1 - \sqrt{1 - f}$, where f is the population frequency of the specificity in question.

Family studies. Population statistics and segregation analysis can support and supplement each other (*6, 8*). The segregation patterns of the cytotoxicity results of the panel sera with the cells of dog families were used to allocate DL-A haplotypes to the individual family members (*23*).

Two antisera or two clusters of antisera that seemed to have an allelic relationship in terms of population statistics were checked in double backcross matings ($1/2 \times -/-$, 1 and 2 being the allelic specificities of the genetic system). No littermates with both or neither of the specificities should be present. No DL-A haplotypes with two components of one series of alleles should occur (absence of "doublets"). Independent specificities can be present in the same littermates from another type of double backcross mating ($1,4/-,- \times -,-/-,-$), 1 and 4 being two specificities belonging to two different series of multiple alleles. The same type of double backcross mating can be used to find support for the concept that two independent series of multiple alleles are located on the same homologous chromosome pair.

These data were not extensive enough for analysis using standard tests for the estimation of linkage (*6*).

The number of negative animals in dog families of mating type $+/- \times -/-$ (a single backcross) were used to determine whether the segregation behaviour of the studied specificities was compatible with the segregation pattern of a dominant Mendelian character (*14*).

NOMENCLATURE

In the future, agreement will have to be made on a standard nomenclature for the antigenic determinants of the DL-A system. In this paper the antigenic determinants have been assigned

arabic numerals, preceded by the initials DL to emphasize that they have been defined on dog lymphocytes. DL 1 is detected by sera 3897 and 3895; DL 2 is detected by serum 7883; DL 3 is detected by serum 7884; DL 4 is detected by sera 7865, 7875, and 7882; DL 5 is detected by sera 7878, *7833*, and 7877; DL 6 is detected by serum *7864*. Serum 7833 (DL 5) and serum 7864 (DL 6) were obtained from mongrel dogs. The others were from beagles.

As of yet, absorption experiments (*24*) have only been performed with serum 3897 (DL 1) and 7865 (DL 4). Leucocytes (10^6) were used per 0.1 ml of serum. Absorptions were carried out with suspensions from 10 unrelated dogs reacting positively with the unabsorbed antiserum. The results indicated that these antisera are probably operationally monospecific.

RESULTS

Figures 1 and 2 show the χ^2 values of independence for 16 antisera arranged in a triangle. Sera were clustered within one specificity if their χ^2s for independence were significantly high. Sera such as 7882 and 7877 were included in the DL 4 and DL 5 clusters, respectively (Fig. 5), even though they were clearly broader than the main specificity. In the event of equivocal results within a cluster, the results of a leading serum were accepted as decisive. Ten other antisera from the panel are not depicted. These sera either gave inconsistent results in the family studies (for example, the cells of the dame and sire were negative and the cells of some of the litter were positive), or included one or more of the specificities in the triangle. A comparison between the results in mongrels and beagles shows that, in general, the same statistical relationship exists between the different antisera in the two dog populations. There are, however, a number of exceptions. The most notable difference is the negative association between DL 5 (sera 7878, 7833, and 7877) and DL 6 (serum 7864) in beagles, which have a high positive association in the mongrel population.

χ^2 Tests for allelism (Figs. 3 and 4) indicate that some sera could have allelic relationships. The possible relationships between the antisera become clearer when the results from Figures 1 and 2 and 3 and 4 are compared (Table 1). Two "allelic" antisera have a negative association when tested for independence and a low χ^2 value for allelism. Two "independent" antisera will have a low χ^2 value for independence and a high χ^2 value for allelism. In Table 1, only one serum/specificity is shown. DL 1, DL 2, and DL 3 seem to be the antigenic determinants of a first series of multiple alleles. The data suggest that DL 4, DL 5, and DL 6 are independent of this first series. The respective χ^2 values for in-

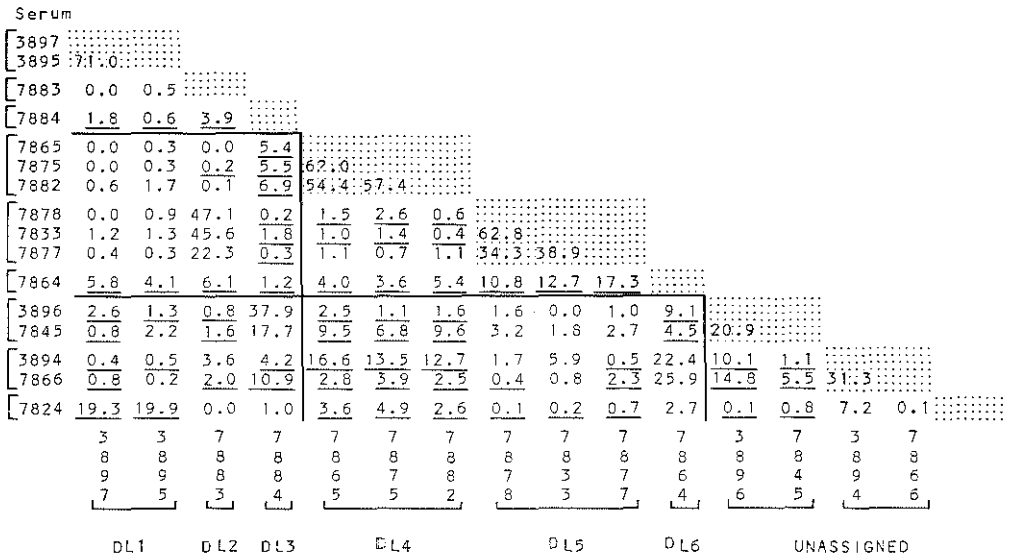

Figure 1. χ^2 Values for independence (1 DF) between serum pairs tested in 77 beagles. Underlined values indicate a negative association.

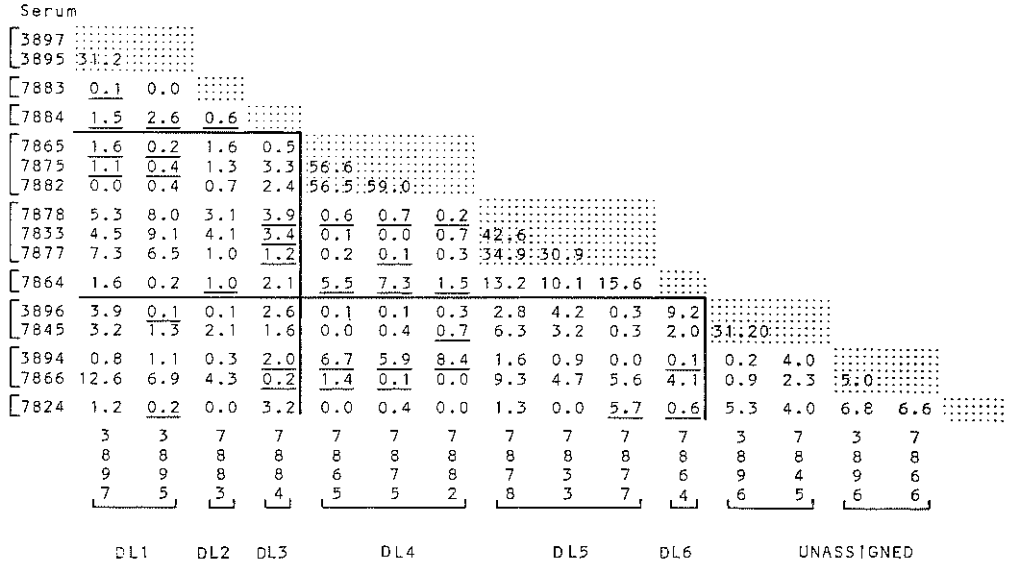

FIGURE 2. χ^2 Values for independence (1 DF) between serum pairs tested in 100 mongrel dogs. Underlined values indicate a negative association.

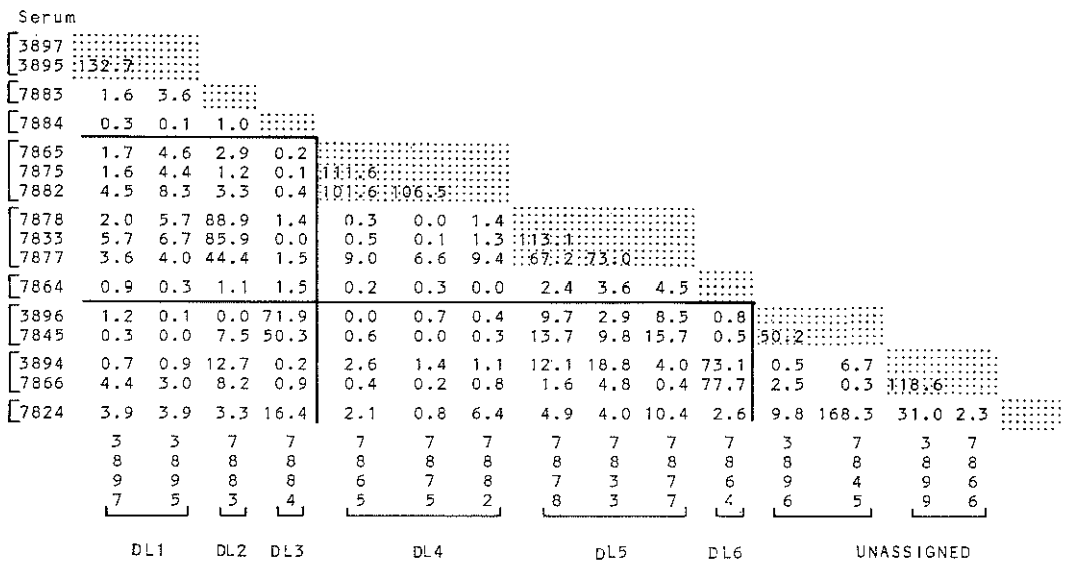

FIGURE 3. χ^2 Values for allelism (1 DF) between serum pairs tested in 77 beagles.

dependence and allelism indicate that DL 4, DL 5, and DL 6 could be the antigenic determinants of a second series of multiple alleles in beagles but not in mongrel dogs.

Table 2 shows the gene frequencies for the six specificities in mongrels and beagles and the differences between the two populations. These gene frequencies were used to calculate the gene frequency of a postulated blank allele(s). They were used to calculate the expected number of different phenotypes per series. The gene frequencies (Table 2) and the family studies (Fig. 5) for the nonassigned antisera are not given here because their analysis is still in a preliminary state.

Table 3 shows that, in the first series, no tri-

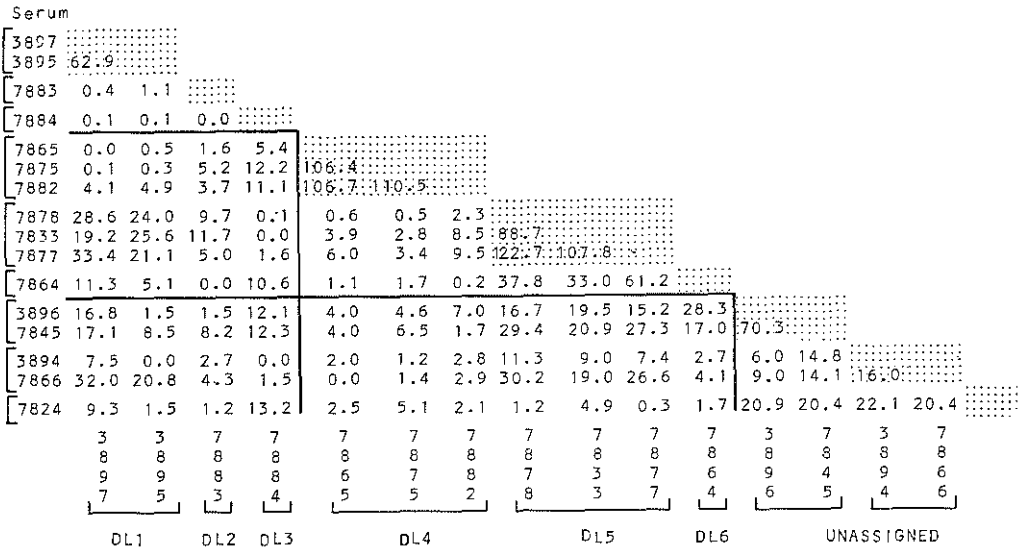

FIGURE 4. χ^2 Values for allelism (1 DF) between serum pairs tested in 100 mongrel dogs.

plets were found and that the observed numbers of different phenotypes in mongrels and beagles agree very well with the null hypothesis of at least 4 allelic specificities. In the second series (Table 4), one mongrel was positive for DL 4, DL 5, and DL 6. In beagles no triplets were found. However, in both mongrels and beagles, a sufficiently good fit of expected and observed results could not be obtained to statistically confirm a second series.

TABLE 1. χ^2 Values of independence and allelism between serum pairs

Sera and specificities	χ^2 Independence[a]		χ^2 Allelism	
	Mongrels	Beagles	Mongrels	Beagles
3897(DL 1)-7883(DL 2)	*0.1*	0.0	0.4	1.6
3897(DL 1)-7884(DL 3)	*1.5*	*1.8*	0.1	0.3
7883(DL 2)-7884(DL 3)	*0.6*	*3.9*	0.0	1.0
7875(DL 4)-3897(DL 1)	*1.1*	0.0	0.1	1.6
7875(DL 4)-7883(DL 2)	*0.4*	*0.2*	5.2	2.9
7875(DL 4)-7884(DL 3)	3.3	*5.6*	12.2	0.2
7878(DL 5)-3897(DL 1)	5.3	0.0	20.6	2.0
7878(DL 5)-7883(DL 2)	8.0	47.1	9.7	88.9
7878(DL 5)-7884(DL 3)	*3.4*	*0.2*	0.1	1.4
7864(DL 6)-3897(DL 1)	1.6	*5.8*	11.3	0.9
7864(DL 6)-7883(DL 2)	0.2	6.1	0.0	1.1
7864(DL 6)-7884(DL 3)	2.1	*1.2*	10.6	1.5
7875(DL 4)-7878(DL 5)	*0.7*	*2.6*	0.5	0.1
7875(DL 4)-7864(DL 6)	*7.3*	*3.6*	1.7	0.3
7878(DL 5)-7864(DL 6)	13.2	*10.8*	37.8	3.6

[a] Italic χ^2 values indicate a negative association.

TABLE 2. Gene frequencies in two dog populations

Specificity	Antisera detecting specificity	Gene frequency (SE)	
		Mongrels	Beagles
DL 1	3897	0.1472 (0.0277)	0.0812 (0.0219)
	3895		
DL 2	7883	0.0541 (0.0187)	0.1088 (0.0270)
DL 3	7884	0.1528 (0.0296)	0.2118 (0.0355)
DL 4	7865		
	7875	0.1044 (0.0225)	0.2478 (0.0404)
	7882		
DL 5	7878		
	7833	0.3195 (0.0337)	0.1571 (0.0295)
	7877		
DL 6	7864	0.2478 (0.0404)	0.3134 (0.0469)

In Figure 5 several examples of segregation in beagle (families 1–5) and mongrel (families 6–7) families are shown. DL-A haplotypes could be allocated using the panel of 26 sera in 61 out of the 70 beagle families and 8 out of the 10 mongrel families. An easy way to haplotype a dog family is to study the serum reactions which are positive with the cells of the sire and negative with the cells of the dame (a paternal backcross). These sera can have two different patterns in a litter, one recognizing the segregation of haplotype A, and the other recognizing the segregation of haplotype B (for example DL 1 and DL 4 in family 2). In a similar way, one

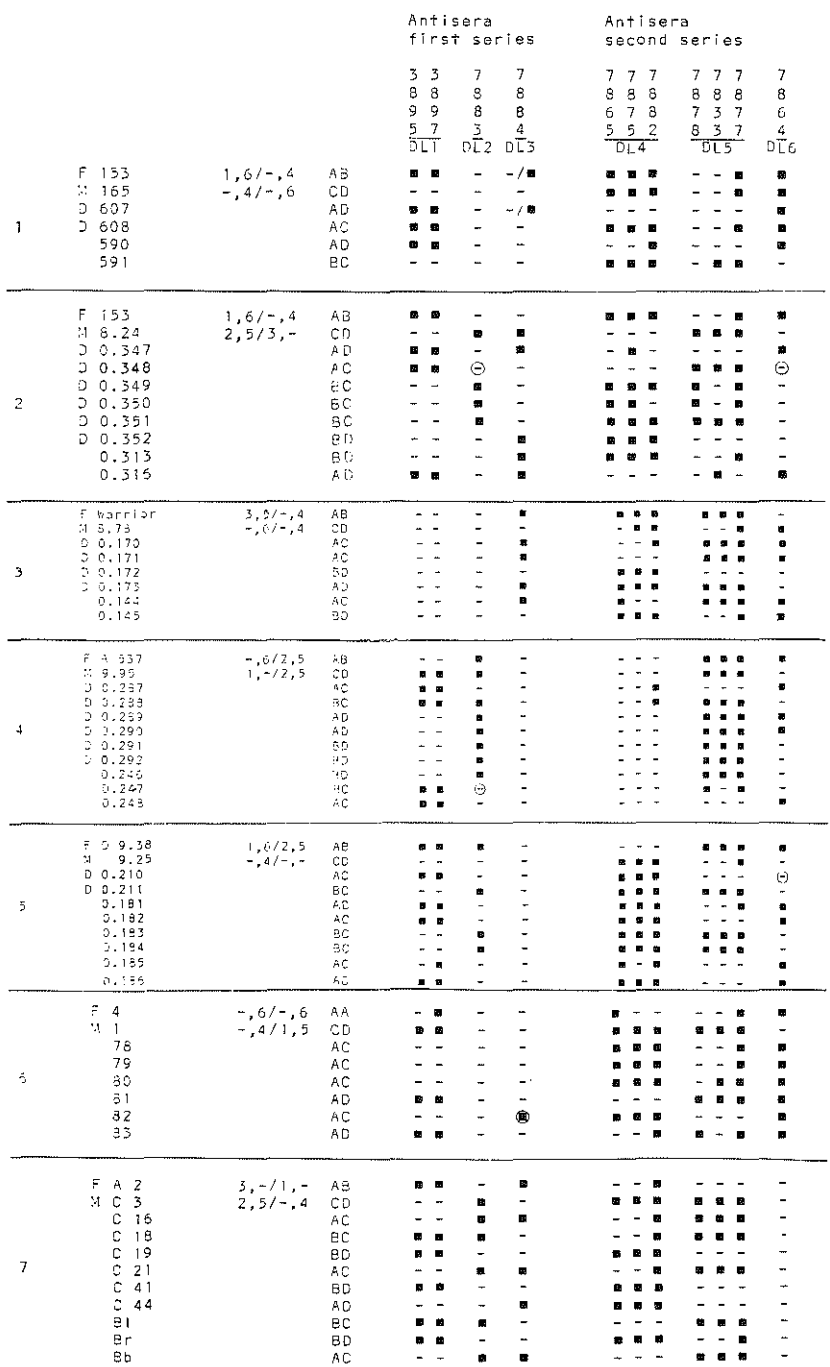

FIGURE 5. Segregation patterns of 6 DL specificities in five beagle (1–5) and two mongrel (6–7) dog families.

can allocate the haplotypes C and D of the dame by studying the sera negative with the cells of the sire and positive with the cells of the dame, a maternal backcross (for example, DL 4 and DL 5 in family 7).

Transplantation experiments have shown that the DL-A haplotypes obtained in this way were meaningful in terms of graft survival. Allografts (skin (*23*), small bowel (*26*), kidney (*27*), heart (*5*), and pancreas (de Gruyl, unpublished data)) from DL-A-identical littermates survived significantly longer than grafts from DL-A-nonidentical littermates. All families in Figure 5 are examples of the two different kinds of double backcross matings described in Material and Methods.

If there are two series of multiple alleles in the DL-A system, their genetic determinants should occur on the same homologous chromosome. In family 3 (Fig. 5) DL 3 from the postulated first series and DL 5 from the postulated second series are part of the same paternal haplotype. The results, which do not fit the rules explained in Material and Methods, are encircled.

TABLE 3. Distribution of DL-A specificities 1, 2, and 3 in 100 random mongrels and 77 beagles

DL specificities			No. of mongrels[a]		No. of beagles[b]	
1	2	3	Observed	Expected	Observed	Expected
+	+	+	0	0	0	0
+	+	−	2	1.58	2	1.38
+	−	+	4	4.47	1	2.69
−	+	+	1	1.64	0	1.80
+	−	−	22	21.75	7	8.71
−	+	−	5	7.23	11	11.10
−	−	+	19	21.91	24	23.32
−	−	−	47	41.42	32	28.00
Total			100	100.00	77	77.00

[a] Mongrels' χ^2_3, 2.27; $0.25 < P < 0.50$.
[b] Beagles' χ^2_3, 4.07; $P > 0.2$.

TABLE 4. Distribution of DL-A specificities 4, 5, and 6 in 100 random mongrels and 77 beagles

DL specificities			No. of mongrels[a]		No. of beagles[b]	
4	5	6	Observed	Expected	Observed	Expected
+	+	+	1	0	0	0
+	+	−	7	6.67	5	5.99
+	−	+	1	5.74	11	11.96
−	+	+	24	15.83	2	7.58
+	−	−	9	7.94	15	15.47
−	+	−	14	31.19	15	8.72
−	−	+	6	22.41	24	21.17
−	−	−	38	10.78	5	6.11
Total			100	100.00	77	77.00

[a] Mongrels' χ^2_3 gives a $P \ll 0.001$; results are not compatible with a series of four multiple alleles.
[b] Beagles' χ^2_3, 9.48; $0.001 < P < 0.025$; results are not compatible with series of four multiple alleles.

TABLE 5. DL-A haplotypes found in unrelated genotyped beagles (80 haplotypes) and mongrels (36 haplotypes)

Specificities on haplotypes of series		No. found
1st	2nd	
DL 1	DL 4	2
DL 1	DL 5	3
DL 1	DL 6	2
DL 1	—	6
DL 2	DL 4	1
DL 2	DL 5	10
DL 2	DL 6	—
DL 2	—	1
DL 3	DL 4	1
DL 3	DL 5	2
DL 3	DL 6	2
DL 3	—	11
—	DL 4	20
—	DL 5	9
—	DL 6	26
—	—	20
Total		116

The reproducibility of the test, the fact that families 1–5 were only tested once, and the fact that some specificities are recognized by only one antiserum raise the possibility that these abberant results in family typing were caused by serological errors. In the 80 dog families studied, one possible example of recombination in the chromosomal DL-A region has been found. The analysis of this beagle family will be reported after more extensive studies.

Table 5 summarizes the DL-A haplotypes found in beagles and mongrels. No doublets were found. Table 6 gives an example of the type of investigation employed to study the possible Mendelian character of the different specificities. The low χ^2 value shows an excellent fit with the null hypothesis that the studied specificity is a dominant Mendelian factor. All the other specificities studied had similar low χ^2 value in this test.

DISCUSSION

In man (*1*), mice (*15, 19*), and probably rhesus monkeys (*3*), the genetic determinants of

TABLE 6. Analysis of No. of negative littermates in families of mating type DL 3+/DL 3− × DL 3−/DL 3−[a]

Litter size	No. of families	No. of negatives		Variance
		Observed	Expected	
2	2	2	2.666	0.444
5	2	3	5.162	2.164
6	1	2	3.048	1.379
7	1	5	3.528	1.667
8	2	8	8.032	3.890
9	1	5	4.509	2.215
Total	9	25 (R_1)	26.945 (E_1)	11.759 (V_1)

[a] $\chi^2(1\ DF) = (R_1 - E_1)/V_1 = 0.33$.

the major histocompatibility system are defined by multiple alleles of two segregant series occupying two closely linked loci or subloci. Our results in canine tissue typing suggest a similar genetic structure for the DL-A system. Nevertheless, other explanations are possible. The methods used in this study have been critically discussed by Andresen et al. (*2*), Mi and Morton (*10*), and Ceppellini et al. (*6*). Most of their comments on numbers of tested subjects, serological inconsistency, and gametic associations or linkage disequilibrium can be applied to the results described here. The evidence for the first series of multiple alleles in the DL-A system appears to be sufficient. Evidence for the second series is still preliminary. Although the analysis of DL 4, DL 5, and DL 6 indicates that there is probably another series of multiple alleles besides the first series, an exact analysis is hampered by the fact that the specificities DL 5 and DL 6 are probably not monospecific. In fact, DL 5 and DL 6 have a high positive association in mongrels and DL 2 and DL 5 are highly positively associated in both dog populations.

Cross reactivity and cytotoxicity-negative absorption-positive phenomena (*28*) have not yet been studied in the dog. They complicate the definition of monospecificity and the determination of the exact number of (sub)loci of a major histocompatibility system. Additional evidence for the existence of two series of multiple alleles will perhaps be aided by the testing of more dogs, planned immunisations, and absorption studies. Only the full analysis of the most promising antisera was given here. The 10 antisera with a higher frequency of positive reactions, which include the shorter specificities described in this article, are particularly interesting since they might provide an experimental model to study the biological significance of systems like the 4a/4b system in humans (*20, 21*). Differences in the gene frequencies of some of the six specificities in beagles and mongrels are perhaps analogous to differences in HL-A gene frequencies in various human ethnic groups (*13*). These results are in contrast to what is known about the gene frequencies of erythrocyte groups in dogs (*17*). The differences could, however, also be explained by the small sample size, genetic drift in a limited population, or selective breeding (*7*).

The biological significance of major histocompatibility loci is not yet known. The dog appears to have a high degree of polymorphism in his major histocompatibility system. The possible existence of two subloci in the DL-A system and of varying DL-A gene frequencies in different dog breeds suggest a high degree of similarity between HL-A and DL-A. Thus, the dog seems suitable for the exploration of current clinical and biological problems associated with histocompatibility genetics.

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REFERENCES

1. Allen, F. et al. 1970. p. 2. *In* P. I. Terasaki (ed.). *Histocompatibility testing 1970*. Munksgaard Copenhagen.
2. Andresen, E.; Baker, L. N.; Rowe, K. E. 1963. Immunogenet. Letter *3:* 15.
3. Balner, H.; van Rood, J. J. 1971. Nature *232:* 121.
4. Balner, H.; Gabb, B. W.; Dersjant, H.; van Vreeswijk, W.; van Rood, J. J. 1971. Nature *230:* 177.
5. Bos, E.; Meeter, K.; Stibbe, J.; Vriesendorp, H. M.; Westbroek, D. L.; de Vries, M. J.; Nauta, J.; van Rood, J. J. 1971. Transpl. Proc. *3:* 155.
6. Ceppellini, R.; Curtoni, E. S.; Mattiuz, P. L.; Miggiano, V.; Scudeller, G.; Serra, A. 1967.

p. 149. *In* E. S. Curtoni, P. L. Mattiuz, and R. M. Tosi (eds.). *Histocompatibility testing 1967*. Munksgaard, Copenhagen.
7. Cohen, C.; Fuller, J. L. 1953. J. Hered. *44:* 225.
8. Dausset, J.; Colombani, J.; Legrand, L.; Fellous, M. 1970. p. 53. *In* P. I. Terasaki (ed.). *Histocompatibility testing 1970*. Munksgaard, Copenhagen.
9. Ivanyi, P. 1970. p. 8. In *Current topics in microbiology and immunology*. Vol. 53. Springer, Heidelberg.
10. Mi, M. P.; Morton, N. E. 1966. Vox Sang. *11:* 434.
11. Palm, J. 1964. Transplantation *2:* 603.
12. Rapaport, F. T.; Hanaoka, T.; Shimada, T.; Cannon, F. D.; Ferrebee, J. W. 1970. J. Exp. Med. *131:* 881.
13. Singal, D. P.; Mickey, M. R.; Terasaki, P. I. 1969. Transplantation *8:* 829.
14. Smith, C. B. A. 1956. Ann. Hum. Genet. *20:* 257.
15. Snell, G. D.; Cherry, M.; Démant, P. 1971. Transpl. Proc. *3:* 183.
16. Snell, G. D.; Smith, P.; Gabrielson, F. 1953. J. Nat. Cancer Inst. *14:* 457.
17. Swisher, N.; Young, L. E. 1961. Physiol. Rev. *41:* 495.
18. Templeton, J. W.; Thomas, E. D. 1971. Transplantation *11:* 429.
19. Thorsby, E. 1971. Europ. J. Immunol. *1:* 57.
20. van Rood, J. J. 1962. Ph.D. Thesis. University of Leiden, Leiden, The Netherlands.
21. van Rood, J. J.; van Leeuwen, A.; Zweerus, R. 1970. p. 93. *In* P. I. Terasaki (ed.). *Histocompatibility testing 1970*. Munksgaard, Copenhagen.
22. Viza, D.; Sugar, J. A.; Binns, R. M. 1970. Nature *227:* 949.
23. Vriesendorp, H. M.; Rothengatter, C.; Bos, E.; Westbroek, D. L.; van Rood, J. J. 1971. Transplantation *11:* 440.
24. Vriesendorp, H. M.; Bos, E.; Rothengatter, C.; de Gruyl, J.; Westbroek, D. L.; Epstein, R. B.; van Rood, J. J. 1971. p. 152. *In* S. Bengmark and A. Olsson (eds.). *Abstracts of the sixth congress of the European Society for Experimental Surgery*. Ab Gambro, Lund, Sweden.
25. Walford, R. L.; Shandron, E.; Troup, C. M.; Zeller, E.; Ackerman, B. 1967. p. 221. *In* E. S. Curtoni, P. L. Mattiuz, and R. M. Tosi (eds.). *Histocompatibility testing 1967*. Munksgaard, Copenhagen.
26. Westbroek, D. L.; Rothengatter, C.; Vriesendorp, H. M.; van Rood, J. J.; Willighagen, R. G. J.; de Vries, M. J. 1971. Transpl. Proc. *3:* 157.
27. Westbroek, D. L.; Vriesendorp, H. M.; Schönherr-Scholtes, Y.; van Urk, H.; Silberbusch, J.; de Vries, M. J. 1971. p. 70. *In* S. Bengmark and A. Olsson (eds.). *Abstracts of the sixth congress of the European Society for Experimental Surgery*. Ab Gambro, Lund, Sweden.
28. Yunis, E. J.; Ward, F. E.; Amos, D. B. 1970. p. 351. *In* P. I. Terasaki (ed.). *Histocompatibility testing 1970*. Munksgaard, Copenhagen.

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CHAPTER 4

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Analysis of the DL-A System in Families and Populations of Healthy and Diseased Individuals

By H. M. Vriesendorp, J. D'Amaro, J. A. van der Does, D. L. Westbroek, and R. B. Epstein

A LARGE body of information is available on major histocompatibility systems of man, rhesus monkey, and small rodents.[1] In our laboratories studies were initiated to obtain a comparable amount of knowledge of dog histocompatibility antigens. In previous reports we have demonstrated that the dog as the above mentioned mammalian species, possesses only one major histocompatibility system (DL-A), possibly consisting of two linked series of multiple alleles.[2,3] The purpose of this paper is to present a progress report on DL-A antigen recognition. The occurence of DL-A antigens in two different dog populations, in dog families and in groups of diseased dogs will be reported and compared to analogous data obtained in human histocompatibility studies.

MATERIALS AND METHODS

Animals

Unrelated mongrel dogs originating from all parts of the Netherlands (approximately 36.200 km^2) were obtained from three different dog handlers. A population of unrelated beagles was selected from animals belonging to six different breeders. When pedigrees were not available, only those animals were included in the analysis, which were born on different days. Blood samples of dogs with leukosis, diabetes mellites, or hemolytic anemia were obtained through the courtesy of the Small Animal Clinic of the State University Utrecht, The Netherlands (Head Professor Dr. G. H. B. Teunisse). Samples of a control group were obtained from animals visiting the same clinic for nonmalignant, non endocrine, mostly surgical disorders. As of yet pathologic studies of biopsy or autopsy material of the leukosis dogs have not been completed. Therefore the more general term "leukosis," will be used instead of the more precise one "malignant lymphoma," since some of the animals might have been suffering from one of the rarer canine leukocyte malignancies.[4]

Test, Sera, Immunogentic Methods

A microcytotoxicity test, with a reproducibility rate of over 90% was used. The test, the production of typing sera and the immunogenetic methods used for the definition of alleles and series have been described previously.[2,3,5]

Seventy to ninety different allo antisera, most of them raised in intrasib immunizations (skingrafts or subcutaneous leukocyte injections) have been used to type the blood samples. All samples were tested twice on two different days, except the ones from leucosis animals, which were typed only once. To investigate the relationships between alleles of different series, gametic associations (Δ) or linkage disequilibrium parameters were computed from random phenotypes using the formula given by Ceppellini.[6] X^2 values of heterogeneity were calculated, following Mather,[7] to measure the significance of the difference in gene frequencies between the two dog populations investigated.

Nomenclature

Twelve specificities have been defined belonging to two linked series of alleles. They have been labeled DL 1–12 (Table 1). It is one of the purposes of a Worshop on Canine Immunogenetics in November 1972 in Rotterdam to reach international agreement on a common nomenclature for DL groups, which can be used instead of local group designations. DL 7 is sometimes labeled DL 7 (long) since the shortest serum of the group has only been available for the second part of the described studies.

Absorptions

Some of the DL 1 and DL 4 sera were used in absorption experiments and appeared to be "operationally monospecific,"[8] using ten different positive cell samples. Absorption studies of the other specificities are in progress.

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Table 1. DL-A Gene Frequencies and △ values in Population of Unrelated Dogs

FIRST SERIES	GENE FREQUENCY	NUMBER OF ANTISERA AVAILABLE FOR GROUP RECOGNITION	Δ VALUES BETWEEN DL GROUPS WITH $P < 0.05$
DL 1	0.0135	8	
DL 2	0.0643	6	
DL 3	0.1928	5	
DL 7	0.0940	5	
DL 9	0.1238	6	
DL 10	0.0940	4	DL 2 - DL 6 + 0.018[a]
Blank	0.4176		DL 3 - DL 12 + 0.018[b]
SECOND SERIES			DL 7 - DL 5 + 0.038[a]
DL 4	0.1302	6	DL 9 - DL 6 + 0.033[c]
DL 5	0.2441	6	DL 9 - DL 5 - 0.031[d]
DL 6	0.0552	4	
DL 8	0.0090	2	
DL 11	0.0552	2	
DL 12	0.0743	2	
Blank	0.4320		

a - $0.001 < P < 0.005$
b - $0.01 < P < 0.025$
c - $P = 0.001$
d - $0.025 < P < 0.05$

RESULTS

Serology

The phenotypes of 112 unrelated mongrel dogs were used to investigate whether the distribution of the groups of the two series obeyed Hardy-Weinberg equilibrium. In both series reasonable fits with expectations based on Hardy-Weinberg equilibrium were obtained. In the first series no triply positive animals were observed. In the second series two triplets were found. In both series the frequency of as of yet unidentified alleles was approximately 0.4. In backcross families the observed segregation ratio's for every DL group were compatible with the ones expected of autosomal dominant Mendelian characters.[9] The amount of polymorphism of the DL-A system which can be identified now is 49 different haplotypes, 1225 different genotypes, and 441 different phenotypes. 29 of the 49 possible different haplotypes have been found in the dog family studies so far. No discordent haplotypes of "doublets" were found. Antigen combinations for which a high positive △ value was computed from the unrelated phenotypes (Table 1) were also found more often in the family studies as a haplotype than haplotypes of antigen combinations for which none significant △ values were found. A strict comparison of △ values from unrelated phenotypes to △ values from family studies is not possible, because of the low number of families studied (45) and the high amount of beagle families among them (30). Three examples of crossing over between the two DL-A series were found. Two occured in a maternal, one in a paternal DL-A chromosome. The recombination frequency in the studied material was $3/410 = 0.007$. Since it has been shown that DL-A disparity is commonly reflected in MLC reactivity,[10] it will be of great interest to perform mixed lymphocyte cultures in these recombinant families. Sera of 17 female dogs, who had given birth to a litter, 6 wks before testing, were tested for the presence of antibodies against the cells of 34 random dogs, including the sire of the litter. Three or 17% appeared to be positive.

Relevance For Transplantation

Figure 1 depicts the influence of prospective DL-A typing on some allograft survivals in littermate donor-recipient pairs. DL-A identical skin,[2] heterotopic small bowel,[11] orthotopic small bowel (L. van Stekelenburg, unpublished observation), pancreas,[12] kidney,[13] orthotopic heart,[14] and uterus (F. J. van Assen, unpublished observation) allografts all survive significantly longer. The longest survivals have been noted in those experiments in which, during transplantation of a DL-A-identical donor organ, bloodtransfusions were given. New experiments have been initiated in an attempt to exclude a random association between blood transfusions and long survival times in DL-A-identical donor–recipient combinations and to explore other factors which could be of importance in this regard. The shortest survival of DL-A identical allografts have been noted in skins, which is analogous to findings in other species. They might be caused by strong, non-DL-A, polymorphic differentiation antigens of skin grafts or occur as a peculiarity of an initially nonvascularized graft. Kidney and small bowel allografts from unrelated DL-A compatible beagles (no "full-house" identical combinations in unrelated donor–recipient pairs have been found yet) survive significantly longer than mismatched organs, though not as long as DL-A-identical grafts from littermates (Ref. 11, Obertop, unpublished data).

Comparison of Two Dog Populations

Table 2 illustrates that the genefrequencies of most DL-A groups in a mongrel dog populuation (N = 114) were significantly different from the ones found in a beagle population (N = 74).

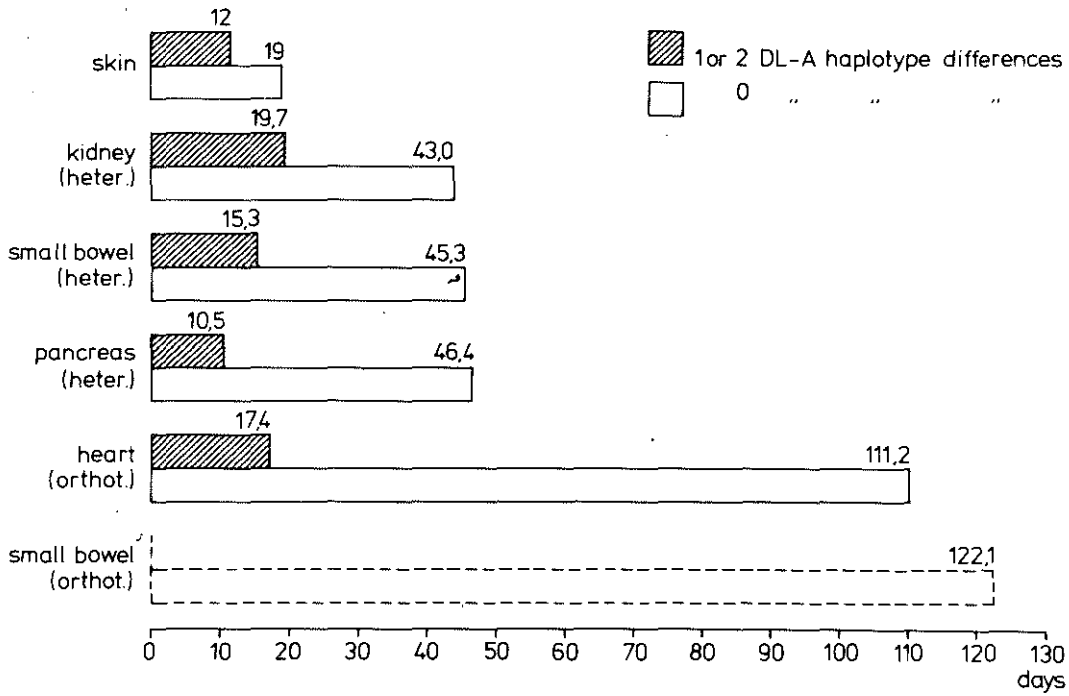

Fig. 1. Mean survival times of different allografts in identical and nonidentical beagle littermates (without immunosuppressive therapy).

Table 2. X^2 Test for Heterogeneity of DL Frequencies of Beagles (N = 74) and Mongrel Dogs (N = 114).

DL GROUP	X^2	SIGNIFICANCE LEVEL
1	1.86	N.S.*
2	10.17	0.002 < P < 0.005
3	4.61	0.025 < P < 0.05
7(long)	8.13	0.001 < P < 0.005
9	22.10	P = 0.0005
4	4.28	0.025 < P < 0.05
5	0.27	N.S.
6	9.32	0.001 < P < 0.005
8	0.48	N.S.

N.S. = not significant

DL-A Groups and Disease

DL-A7 (long) appears to occur more often than expected in leucosis dogs. DL-A 3 and DL-A 7 (long) are more frequent in dogs suffering from diabetes mellites (Table 3). The significance of these findings disappears however, when the *p* values are corrected for the number of comparisons made, as suggested by Bodmer. Future studies will have to determine the real significance of the correlations found.

Linkage

Independent segregation of the DL-A system and the dog coat color loci B, E, S and T (15) have been found. This excludes close linkage between these coat color systems and the DL-A system.

DISCUSSION AND CONCLUSIONS

A striking similarity between the human HL-A system and the canine DL-A system has been observed. Both are extremely polymorphic and consist of two series of multiple alleles. The recombination frequency between the two series in the two species is similar (0.007 compared to 0.008.[16]) Roughly the same percentage of postpartum sera are found to be positive for alloantibodies in both species.[17] Some haplotypes of major histompatibility antigens occur more often than would be expected on a basis of random associations between antigens in both species. This linkage disequilibrium between antigens of the two series can be explained either by recent mutations in the DL-A and HL-A system, either by selectional forces favouring special antigen combinations.[1] The observed variations in DL-A genefrequencies between the two dog populations are comparable to the ones found between different human ethnics groups. The relevance of the DL-A system for histocompatibility has been clearly demonstrated in related donor-recipient pairs and also in as of yet small series of unrelated animals. Obviously the suggestive correlations, which have been observed between certain DL-A antigens and diseases should be confirmed in further studies. If this appears to be the case, new investigations into for example leukosis susceptibility might be profitable.

The observed correlations in dogs are again comparable to human data in which several tentative correlations between special diseases and HL-A antigens were found.[1]

Table 3. Significance Levels* of Difference of DL-A Gene Frequencies in Individuals With Special Diseases Compared to a Control Group (N = 51)

DL GROUP	LEUCOSIS (N=12)	DIABETES MELLITES (N=21)	AUTOIMMUNE HEMOLYTIC ANEMIA (N=8)
1	0.81	0.72	0.86
2	0.21	0.28	0.34
3	$0.05 < P < 0.1$	$0.01 < P < 0.05$	0.23
7(long)	$0.005 < P < 0.01$	$0.01 < P < 0.05$	0.20
9	$0.5 < P < 0.75$	0.14	0.08
10	$0.10 < P < 0.25$	0.26	0.40
4	$0.05 < P < 0.1$	0.02	0.30
5	$0.5 < P < 0.75$	$0.1 < P < 0.25$	0.05
6	0.39	0.26	0.75
8	0.19	0.08	1.
11	0.43	0.38	0.55

*Determined with X^2 test (one degree of freedom) or Fisher's exact test on 2 x 2 table.

The striking similarities, which have been found between the DL-A and the HL-A system make the dog a very appropriate animal for future studies into clinical and biologic problems of histocompatibility research in a randomly bred species.

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REFERENCES

1. Bodmer, W. F.: Nature 237:139, 1972.
2. Vriesendorp, H. M., Rothengatter, C., Bos, E., Westbroek, D. L., and van Rood, J. J.: Transplantation 11:440, 1971.
3. Vriesendorp, H. M., Epstein, R. B., D'Amaro, J., Westbroek, D. L., and van Rood, J. J.: Transplantation. 14:299, 1972.
4. Loeb, W.: *In* Medway, W., Prier, J. E., and Wilkinson, J. S. (Eds.): A Textbook of Veterinary Clinical Pathology. Baltimore, Williams & Wilkens, 1969, p. 335.
5. Vriesendorp, H. M., Bos, E., Rothengatter, C., de Gruyl, J., Westbroek, D. L., Epstein, R. B., and van Rood, J. J.: Europ. Surg. Res. 3:286, 1971.
6. Ceppellini, K., Curtoni, E. S., Mattiuz, P. L., Miggeano, V., Scudeller, G., and Serra, H.: *In* Curtoni, E. S., Mattiuz, P. L., and Tosi, R. M. (Eds.): Histocompatibility Testing 1967. Copenhagen, Munksgaard, 1967, p. 1949.
7. Mather, K.: The Elements of Biometry. London, Methuen, 1967.
8. Walford, R. L., Shandron, E., Troup, C. M., Zeller, E., Ackerman, B.: *In* Curtoni, E. S., Mattiuz, P. L., and Tosi, R. M. (Eds.): Histocompatibility Testing 1967. Copenhagen, Munksgaard, 1967, p. 221.
9. Smith, C. B. A., Ann. Hum. Gen. 20:257, 1956.
10. van der Does, J. A., Epstein, R. B., Walker, W., and van Rood, J. J.: J. Exp. Med. (In press).
11. Westbroek, D. L., Rothengatter, C., Vriesendorp, H. M., and van Rood, J. J.: Europ. Surg. Res. 2:401, 1970.
12. de Gruyl, J., Westbroek, D. L., Dijkhuis, C. M., Vriesendorp, H. M., MacDicken, I., Verschoor, L., and Hulsmans, H. A. M.: Transplant. Proc. 5:000, 19 .
13. Westbroek, D. L., Silberbusch, J., Vriesendorp, H. M., van Urk, H., Roemeling, H. W., Schönherr-Scholtes, Y., and de Vries, M. J.: Transplantation. 14:582, 1972.
14. Bos, E., Meeter, K., Stibber, J., Vriesendorp, H. M., Westbroek, D. L., de Vries, H. J., Nauta, J., and van Rood, J. J.: Transplant. Proc. 3:155, 1972.
15. Little, C. C.: The Inheritance of Coat Colors in Dogs. Ithaca, N.Y., Comstock, 1957.
16. Svejgaard, A., Bratlie, A., Hedin, P. J., Högman, C., Jersild, C., Kissmeyer-Nielsen, F., Lindblom, B., Lindholm, A., Löw, B., Messeter, L., Möller, E., Sandberg, L., Staub-Nielsen, L., and Throsby, E.: Tissue Antigens 1:81 (1971).
17. van Rood, J. J.: Thesis, 1962.

CHAPTER 5

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Joint Report of 1st International Workshop on Canine Immunogenetics

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The mongrel dog is an outbred species which lives in close proximity to man. It shares the human habitat, diet and exposure to microbiological, physical and chemical pathogens. Many human diseases have a canine equivalent (Cornelius 1969). The dog could therefore provide a good experimental model to study these diseases. It could also be useful for the extrapolation of new biological concepts, developed in inbred murine strains, to a readily available outbred species before they are applied to the human. In this respect the dog could be of benefit for studies in transplantation and tumor immunology, immunopathology and population genetics if sufficient "basic" parameters such as histocompatibility antigens, red cell antigens and other genetical markers could be determined in this species. It is felt that a joint effort by research workers engaged in these fields was optimally suited to the assessment of the current state of our knowledge of the dog and to the determination of important future lines of research. Therefore a workshop and symposium on canine immunogenetics was held at the Medical Faculty in Rotterdam, The Netherlands from November 5–16, 1972. The Department of Immunohaematology of the Leyden University Medical Centre and the Laboratory of Experimental Surgery of the Medical Faculty Rotterdam shared the organizational responsibilities. The meetings were sponsored by the International Transplantation Society.

This is a report of the preliminary analysis of the data obtained during this workshop. The genetic systems which can currently be analyzed in dogs by immunological or electrophoretic techniques will be described and discussed. It appears that several important polymorphic systems such as lymphocyte antigens, erythrocyte antigens, red and white cell enzymes, serum proteins and digestive tract antigens can be reasonably well defined in dogs. Immunoglobulin and immune response markers were not studied during this workshop. They remain some of the most important still unidentified genetic markers in the dog. This report will be followed by (1) a discussion of the most evident applications of this new knowledge and (2) abstracts of papers given during the symposium.

Material and Methods

The *investigators* and the *systems* which they studied during the workshop are listed in Table 1.

Dogs: 100 unrelated mongrel dogs (60 males and 40 females) were obtained from three different dog handlers, who collected animals from various parts of the Netherlands (approx. 36,200 km^2). Physical examination placed most animals between 2 and 5 years of age. Eighteen families with a total of 101 pups were available for testing. All parents, except 4 sires which were unknown, were included in the random sample. Fifteen families were from mon-

Table 1

Genetic systems investigated during workshop

System	Investigators
DL–A	Albert, Cabasson, van der Does, Epstein, Erikson, Flad, Hammer, Largiader, von Loringhoven, Saison, Serrou, Schnappauf, Templeton, Uhlschmidt, Vriesendorp
Tumor sera	Epstein
Xenograft sera	Hammer
Lymphocytotoxins recognizing factors other than DL–A	Lang, Albert, Erikson, Flad, von Loringhoven, Schnappauf, Templeton, Vriesendorp (sera supplied by Lang and Cabasson)
Mixed lymphocyte cultures	van der Does, Templeton
Red cell antigens	Bull, van der Does, Swisher, Vriesendorp, Zweibaum
Digestive groups	Zweibaum
Red cell enzymes	Los, Meera Khan, Saison
White cell enzymes	Los, Meera Khan
Serum proteins	Bernini, Saison
Auto immune antibodies	Feltkamp
ABO antibodies	Vriesendorp, Zweibaum

grel dog, two from beagle and one from Labrador parents. No neonatal deaths have occurred. The sex ratio (male/female pups) was 1.5:1, which was the same as in the sample of unrelated dogs (60:40). Reports of high sex ratios in dogs have been published previously (for a review see Burns & Fraser 1966). The 100 unrelated dogs and 80 pups from the 18 families were tested by most workshop participants. Those dogs and 21 additional pups from the same families were investigated for red and white cell enzyme polymorphisms, for digestive group antigens and their "natural occurring" antibodies and for DL–A groups by a restricted number of sera (The Rotterdam-Leyden sera). Albumen polymorphism and the presence of autoimmune antibodies were investigated in a limited number of workshop dogs, 96 and 169 animals respectively.

Methods: A manual of methods used during the workshop is available upon request from D. L. Westbroek, Laboratory for Experimental Surgery, Medical Faculty Rotterdam, The Netherlands. Briefly, a one or two stage microcytotoxicity test was used for lymphocyte typing. Red cell typing was performed with a macroscopically read droplet method, employing fresh autologous serum as cell suspension medium. All reagents were used in hemagglutination, except the CEA1 (old A) and CEA2 (old A1) reagents which were also read for hemolysis. An indirect antiglobulin (Coombs) test was performed with those cells which were negative in hemagglutination and hemolysis for the latter two reagents. The presence of digestive tract antigens and their "natural occurring" antibodies was determined with an indirect immunofluorescent technique using previously selected and defined test sera and a panel of dogs with established digestive tract antigens. Antibodies against human ABO antigens were sought for in absorption tests or comparative titration studies. "Autoimmune" antibodies in dog sera against nuclear factor, smooth muscle, mitochondriae or gastric parietal cells were sought using a 1-to-20 serum dilution in an indirect immunofluorescent technique on rat kidney and gastric tissue.

Analysis of results: The test results of the unrelated animals were analyzed with the help of computer programs. In one group of programs (so called "cluster" and "triangle" programs) results of all the different reagents/techniques were compared pairwise in two-by-two contingency tables (van Rood & van Leeuwen 1963).

Chi squares for independence and allelism (Andresen et al. 1963) and correlation coefficients were calculated. Gene frequencies were determined by the square root method, $GF = 1 - \sqrt{(1 - f)}$, in which f is the population frequency of the factor under investigation. A second program, developed by J. D'Amaro and G. J. v. d. Steen called CHECK, was used to test the population genetics of defined DL groups. Gene frequencies were calculated using the maximum likelihood method (Yasuda & Kimura 1968). Checks on Hardy-Weinberg equilibrium were also made. Linkage disequilibrium parameters or deltas were calculated between the factors of the two segregant series of the DL–A system (Mattiuz et al. 1970).

Family and linkage studies for this report were done manually. A more advanced computer analysis such as a factor analysis (see Albert et al., abstract 5) and a search for the occurrence of loose linkage between markers will be done in the near future and will be the subject of a second paper on the workshop data.

Results

DL–A

Serology: 419 different sera were used in the one or two stage microcytotoxicity test. The latter was the more sensitive. The reproducibility rate of the test was not directly investigated during the workshop. Previous experience has shown that it is approximately 90 %. This is in accordance with the best positive associations found between sera from two different investigators (So1178, a Seattle serum and S14032, a Rotterdam serum) in the workshop, which showed a total of 11 discrepant (—/+ and +/—) results.

Computer analysis 1: From the printouts of the "cluster" and "triangle" programs a search was made for positively correlated sera from *different* investigators. Most of the previously defined DL clusters (Vriesendorp et al. 1972a, 1973) were confirmed by the workshop participants (Table 2). All clusters which did not include DL specificities had high population frequencies except one, which became the DL14 cluster. In the light of the available knowledge on the dog and on other species, a high population frequency for a serum is commonly associated with the presence of antibodies against more than one antigen.

Nomenclature: Since most of the DL group were confirmed and had the lowest population frequencies (and thus a higher probability of being monospecific), it was agreed to use the Rotterdam-Leyden nomenclature until the next workshop, when perhaps more definite symbols can be assigned to antigens by using a DL–A prefix followed by a number, instead of the current DL number. The Rotterdam-Leyden groups number from DL 1 to DL 13. The new cluster found in the workshop with a low population frequency (S 01005, S 010024, and S 01042) has been named DL 14. Absorption experiments for all DL groups are in progress.

Computer analysis 2: The program CHECK tested the fit of the specificities, DL 1, 2, 3, 7, 8, 9, 10 and 4, 5+13, 5—13, 6, 11, 12, 14 as two separate segregant series of alleles. The antigen DL 5+13 was assigned as positive, when both DL 5 and DL 13 were positive. The antigen DL 5 —

Table 2

Two × two tables of results of single workshop sera and previously reported DL groups

DL group	Number of Rotterdam-Leiden sera available for group recognition	Workshop serum	Cells of two × two table			
			−/−	−/+	+/−	+/+
DL 1	4	S 01130	85	9	0	3
DL 2	6	S 01146	52	36	1	9
DL 3	4	S 01159	18	30	5	46
		S 03011	11	35	0	52
		S 10008	36	10	11	41
DL 4	6	S 01036	47	23	6	23
		S 01051	57	13	1	28
		S 01056	53	17	4	25
		S 01078	52	17	6	22
		S 01091	52	16	8	22
		S 01129	53	15	7	23
		S 01178	64	5	4	25
		S 03012	50	20	6	23
		S 04006	29	39	0	28
DL 5	6	S 01033	33	23	3	40
		S 01035	26	30	5	38
		S 01039	28	27	3	37
		S 07019	31	24	4	40
		S 07020	28	17	3	38
DL 6	4	S 01053	75	12	4	8
		S 03016	64	22	2	10
		S 07004	60	26	1	10
DL 7	5	S 01290	43	39	3	15
		S 01147	62	6	8	12
		S 03014	47	38	5	11
DL 8	3	S 03009	85	9	0	5
DL 9	6	S 01051	52	25	6	15
		S 01057	55	22	3	18
		S 01058	62	17	7	14
		S 01128	64	22	3	17
		S 01129	58	18	1	21
		S 03003	59	18	4	17
		S 07002	61	15	3	18
DL 10	5	S 03004	42	35	1	21
		S 07027	60	16	7	15
DL 11	2 (extra reactions of DL 2 sera)	S 07024	44	30	5	20
		S 01150	63	10	9	13
DL 12	2 (extra reactions of DL 9 sera)	S 03012	47	31	8	13
		S 03014	42	33	8	13
		S 07011	42	37	7	12
DL 13	4	none	–	–	–	–

Table 3

Maximum likehood estimates of gene frequencies of DL groups

	'First' series		'Second' series
DL 1	0.0152	DL 4	0.1842
DL 2	0.0517	DL 5 + 13	0.1629
DL 3	0.3022	DL 5 − 13	0.1002
DL 7	0.1125	DL 6	0.0564
DL 8	0.0259	DL 11	0.1507
DL 9	0.1242	DL 12	0.1086
DL 10	0.1143	DL 14	0.0350
Blank	0.2540	Blank	0.2020

13 was assigned as positive, when DL 5 was positive and DL 13 was negative.

The maximum likelihood estimates of the gene frequencies are listed in Table 3. Random phenotypes of DL groups of the "first" segregant series are compatible with Hardy-Weinberg equilibrium (Table 4). No triplets were found. The sample is small, however, and no definite conclusions can be drawn. Table 5 shows the observed and expected phenotypes of DL groups of the "second" segregant series. Two triplets were found (DL 4, DL 5 — 13, DL 11 and DL 4, DL 5 — 13, DL 12).

Table 4

100 random phenotypes of DL groups of first series

Group 1	Group 2	Observed	Expected	χ^2	
DL 1	DL 2	0	0.156	1)	
DL 1	DL 3	1	0.917	1)	
DL 1	DL 7	0	0.341	1)	
DL 1	DL 8	0	0.078	1)	
DL 1	DL 9	1	0.377	1)	
DL 1	DL 10	0	0.347	1)	
DL 1	Blank	1	0.793	1)	
DL 2	DL 3	3	3.123	0.002	1)
DL 2	DL 7	1	1.162	2)	
DL 2	DL 8	1	0.267	2)	
DL 2	DL 9	1	1.283	2)	
DL 2	DL 10	1	1.181	2)	
DL 2	Blank	3	2.891	0.006	2)
DL 3	DL 7	9	6.799	0.712	
DL 3	DL 8	0	1.564	3)	
DL 3	DL 9	9	7.509	0.295	
DL 3	DL 10	10	6.910	1.380	
DL 3	Blank	20	24.486	0.822	
DL 7	DL 8	0	0.582	3)	
DL 7	DL 9	1	2.795	3)	
DL 7	DL 10	3	2.572	1.643	3)
DL 7	Blank	7	6.979	0.000	
DL 8	DL 9	0	0.643	4)	
DL 8	DL 10	1	0.591	4)	
DL 8	Blank	3	1.381	4)	
DL 9	DL 10	3	2.840	0.435	4)
DL 9	Blank	8	7.854	0.002	
DL 10	Blank	4	7.114	1.363	
Blank	Blank	9	6.450	1.007	

Total 7.673 (d.f. = 4)
$0.1 < P < 0.2$

1) 2) 3) 4) indicate that results are pooled to obtain sufficient expectations for analysis.

The P value for this χ^2 value is not compatible with a series of alleles in Hardy-Weinberg equilibrium. When DL 12 was not included in this analysis a χ^2 value with a P between 0.1 and 0.05 was obtained. Omitting DL 4 or DL 5 — 13 did not improve the fit.

Family studies: In the 18 families studied, no discordant haplotypes with two or more DL groups from one series were found. DL groups of the same series were inherited in repulsion. DL groups from the two series segregated as haplotypes. The most common DL haplotypes in mongrel dogs are

Table 5

100 random phenotypes of DL groups of second series

Group 1	Group 2	Observed	Expected	χ^2
DL 4	DL 5 − 13	4	6.001	0.667
DL 4	DL 5 + 13	1	3.691	1)
DL 4	DL 6	1	2.078	2.462 1)
DL 4	DL 11	5	5.552	0.054
DL 4	DL 12	8	4.001	2)
DL 4	DL 14	3	1.289	6.158 2)
DL 4	Blank	11	10.836	0.002
DL 5 − 13	DL 5 + 13	0	3.263	3)
DL 5 − 13	DL 6	4	1.837	0.237 3)
DL 5 − 13	DL 11	9	4.908	4)
DL 5 − 13	DL 12	5	3.538	3.650 4)
DL 5 − 13	DL 14	0	1.140	5)
DL 5 − 13	Blank	8	9.233	0.164
DL 5 + 13	DL 6	1	1.130	5)
DL 5 + 13	DL 11	4	3.019	0.015 5)
DL 5 + 13	DL 12	3	2.176	6)
DL 5 + 13	DL 14	0	0.701	6)
DL 5 + 13	Blank	9	5.050	3.088
DL 6	DL 11	0	1.700	6)
DL 6	DL 12	1	1.225	0.560 6)
DL 6	DL 14	2	0.394	7)
DL 6	Blank	2	2.597	7)
DL 11	DL 12	1	3.273	0.255 7)
DL 11	DL 14	2	1.054	8)
DL 11	Blank	7	8.358	0.220
DL 12	DL 14	0	0.760	8)
DL 12	Blank	3	5.567	1.183
DL 14	Blank	0	1.536	8)
Blank	Blank	10	4.000	2.807 8)

Total 21.531 (d.f. = 7)
$0.01 < P < 0.001$

Total χ^2 of the same analysis without DL 12 = 10.319 (d.f. = 5)
$0.05 < P < 0.1$

1) 2) 3) etc. indicate that results are pooled to obtain sufficient expectations for analysis.

Table 6

Common combinations of DL groups

Group first series	Group second series	Observed	Expected	χ^2	Delta
DL 2	DL 5 – 13	5	1.554	7.635[c]	0.013
DL 2	DL 6	4	0.580	20.123[a]	0.016
DL 3	DL 11	20	8.945	13.662[a]	0.048
DL 7	DL 5 + 13	10	2.100	29.709[a]	0.037
DL 7	DL 12	7	2.347	9.220[b]	0.017
DL 9	DL 4	13	4.148	18.882[a]	0.039
DL 9	DL 6	5	1.386	9.414[b]	0.014
DL 10	DL 5 – 13	12	3.539	20.227[a]	0.038

[a] $P < 0.0005$
[b] $0.001 < P < 0.005$
[c] $0.005 < P < 0.01$

listed in Table 6. Only those combinations which show a delta value > 0.01 and a $P < 0.05$ are indicated. A possible example of crossing over between two paternal haplotypes was encountered in workshop family 004 (see also MLC paragraph).

Possible cross reacting DL groups: Several of the sera in the clusters DL 3, DL 7 or DL 10 reacted often positively with cells not carrying the group recognized by the cluster. The extra reactions always occurred with cells positive for DL 3, 7 or 10. This could be explained by the existence of antibodies which crossreact with DL 3, DL 7 and DL 10, all of which are from the first segregant series. This hypothesis is supported by the fact that most of those sera were raised using donor-recipient combinations which differed in only one antigen per series (i.e. littermates with one DL–A haplotype difference). Appropriate absorption studies to investigate this are in progress. Previous reports (Vriesendorp et al. 1972a, 1973) have included DL 5 in the second segregant series of the DL–A system. Data obtained during the workshop showed that DL 5 could be split into at least two parts. Part one (DL 5 + 13) consisted of reactions with cells positive for DL 5 and DL 13. Part two (DL 5 — 13) consisted of cells positive for DL 5 and negative for DL 13. Both parts appear to belong to the second segregant series. This could be an example of cross reactivity between DL groups in the second segregant series.

Lymphocytotoxins recognizing factors other than DL–A

Several sera consistently showed segregation patterns in families which were different from the ones observed for DL haplotypes. The results of two of these sera are summarized in Table 7. Serum S 14113 in the shortest part of a cluster of positively associated sera from different centers (S 04003 Ulm, S 01110 Seattle, S 14113 Columbus). The correlation between S 14060 and S 14113 in unrelated animals suggests an allelic relationship since the sera are negatively correlated (χ^2 for independence 4.04) and have a low χ^2 for allellism, 0.99. In the 18 workshop families no double backcross matings were available to check this hypothesis of allelism.

Table 7

Lymphocytotoxins, determining factors other than DL–A

Serum number	Source	Gene frequency	No DL–A segregation (No. of families)	Number of expected positive siblings/ Number of observed positive siblings
S 14060	Cabasson (Montpellier, France)	0.1652	4/5	26/30
S 14113	Lang (Columbus, U.S.A.)	0.1125	6/6	22/22

Sera from dogs with regressing Transmissable Venereal Tumor (TVT) (see Epstein & Bennet, abstract 10)

Four early regressor sera were included in the workshop analysis. All appeared to include DL 6 and DL 7. This confirms the findings of Epstein & Bennet that tumor survival is influenced by the DL–A phenotypes of the recipients and provides indirect evidence for the presence of DL 6 and DL 7 on TVT cells.

Dog and Fox

During the workshop two samples of fox blood were tested for DL groups. Earlier findings (Vriesendorp 1972b) were confirmed. Fox cells show polymorphism with dog allo antisera from prolonged immunization schedules, especially in the more sensitive two stage cytotoxicity test. No positive reactions were found with sera with a low population frequency in a one stage test.

Two sera in the workshop were produced by dogs which had rejected a fox skin graft (see Hammer, abstract 11). Both showed reactivity patterns in the one stage cytotoxicity test with dog lymphocytes which included DL groups. They were not tested in the two stage cytotoxicity test.

Mixed Lymphocyte Cultures (MLC)

MLCs in dogs follow the patterns of human, rhesus monkey and mouse MLCs, since lymphocytes of sibs, which are identical for the serologically defined major histocompatibility antigens, do not stimu-

Table 8

DL–A haplotypes of workshop family 004

Dog	DL groups					DL genotypes	DL hapoltypes
	DL 3	DL 5	DL 7	DL 10	DL 12		
00400 (dame)	+	+	–	+	–	3,5/10,5	C/D
00401 (sire)	–	+	+	+	+	7,12/10,5	A/B
00402	+	+	–	+	–	3,5/10,5	B/C
00403	+	+	+	–	–	3,5/7,*5*	*A*/*B*/C
00404	–	+	+	+	+	10,5/7,12	A/D
00405	+	+	–	+	–	3,5/10,5	B/C

Repeatedly no stimulation in all possible two-way MLCs between 00402, 00403 and 00405.

late (Templeton & Thomas 1971, van der Does et al. 1972).

The technique, however, is more difficult and less reproducible in the dog. Two exceptions to serotypic and MLC identity have been found, one by Templeton, where there was no stimulation between sibs that were serologically *non identical,* and the other in a workshop family which had an offspring in which a recombination between the first and second series occurred. The difference for the antigens DL 7 and DL 10 between this dog (a, b/c) and sibs (b/c) repeatedly did not give rise to stimulation in MLC (Table 8). These observations could represent the segregation of a locus (or loci) linked to, but distinct from, DL–A which determines MLC reactivity.

Autoimmune Antibodies in Dogs

No antibodies against gastric parietal cells and mitochondria were found. Table 9 lists the positive findings for anti nuclear and anti smooth muscle antibodies. The sex ratio in that group of dogs is not significantly different from the one found in the dogs without autoimmune antibodies. Digestive tract group X appeared to be present in all dogs with antinuclear antibodies. CEA 2 (old erythrocyte antigen A 1) and CEA 8 (old He) were present in 8 out of 9 and 7 out of 9 animals carrying autoimmune antibodies, respectively. DL 12 was the only DL group which occurred significantly more frequently in these dogs, compared to a control group of unrelated animals (N = 72) without autoimmune antibodies ($\chi^2 = 5.12$, P (corrected for the number of comparisons made) between 0.05 and 0.025).

Canine Erythrocyte Antigens

Serology

Twelve sera were used to define the currently known canine erythrocyte groups in the workshop dogs (see S. N. Swisher & Young 1961, Swisher & Bull, abstract 2).

Eleven previously unanalyzed anti canine erythrocyte sera from other investigators were also included. Their results ap-

Table 9

Presence of autoimmune antibodies in sera of workshop dogs

Dog	Sex	Antinuclear antibodies	Anti smooth muscle antibodies	DL groups	
				First series	Second series
C 04000	female	+	−	3, 10	4, 12
C 07401	male	±	−	3, −	−, −
C 03101	male	±	−	3, 10	−, 12
C 04101	male	±	+	−, −	4, 12
C 00705	female	−	+	3, 7	5−13, 12
C 00704	male	−	+	3, −	−, 12
C 00903	male	−	+	−, 10	4, 12
C 03901	male	−	+	3, 10	−, 11
C 01704	male	−	+	−, 9	6, −
C 01705	female	−	±	3, 9	6, −
C 05301	male	−	±	3, 10	5+13, −
C 00106	male	−	±	3, 9	4, −
C 04800	female	−	±	2, 3	5−13, 12

Sex ratio male/female = 9 : 4

peared to fit in with the already known groups. Some were polyspecific and probably also contained extra antibodies against undefined canine erythrocyte antigens.

Population Frequency and Family Studies

Table 10 indicates nomenclature and population frequencies of the Canine Erythrocyte Antigens (CEA) studied in this workshop. CEA 3 (old B) was not found in this sample of Dutch unrelated dogs. CEA 4 (old C) and CEA 6 (old F) had a high population frequency. They did not segregate in the workshop families. CEA 1, 2, 5, 7 and 8 (old A 1, A 2, D, Tr and He) segregated as codominant Mendelian factors.

ABO related systems in dogs: As early as 1910, von Dungern & Hirschfeld reported the occurrence of anti human A and anti human B antibodies and "ABO like" antigens in many species including the dog; CEA 7 (old Tr) was closely correlated with the presence of digestive tract group A (previously shown to be human ABO A related, Zweibaum et al. 1966) and the absence of anti human A in the dog serum. A rabbit antiserum against canine digestive group A substance was produced by Zweibaum. Its reactions with dog erythrocytes were almost completely included in the reaction pattern of dogs positive for digestive tract group A and were positively correlated with CEA 7.

Digestive Tract Groups

A tri-allelic system for antigens within and secreted by mucous cells of the canine digestive tract has been described by Zweibaum & Steudler 1969 (see also Zweibaum & Feingold, abstract 1). "Natural occuring" antibodies against the alleles A, X or Y have been instrumental in the discovery of this system. These alleles appear to have the same negative association in the unrelated Dutch mongrel dogs as have been found in earlier studies in France. Their gene frequencies were also similar. The segregation in the families was in concordance with the genetical hypothesis of three alleles belonging to one system. The relationship of this system with the human

Table 10

Nomenclature[a] and population frequency of canine erythrocyte antigens

Nomenclature		Population frequency in 100 unrelated Dutch mongrel dogs
New	Old	
CEA 1	A	66%
CEA 2	A_1	51%
(CEA 1+; CEA 2−)	A_2	15%
CEA 3	B	0%
CEA 4	C	97%
CEA 5	D	17%
CEA 6	F	97%
CEA 7	Tr	47%
CEA 8	He	39%

[a] The nomenclature for blood groups in dogs is confusing. For example the A antigen in dogs is unrelated to the human A, while the canine Tr appears to have some relation with the human A (Bowdler et al. 1971). Tentative new designations are given here. Newly discovered antigens will be numbered 9, 10 etc.

erythrocyte ABO system and the canine erythrocyte antigen CEA 7 (old Tr) has been described above. No correlations with other CEAs or DL-groups were found.

Red Cell and White Cell Enzyme Polymorphisms

The following enzyme systems were tested for electrophoretically detectable variations in the red and white cells of the workshop dogs by the Leyden group. Adenosine deaminase (ADA), adenylate kinase (AK), glucose phosphate isomerase (GPI), cytoplasmic isocitrate dehydrogenase (C–IDH), lactate dehydrogenase A (LDH–A), LDH–B, cytoplasmic NAD-dependent malate dehydrogenase (C–NAD–MDH), mitochondrial (M) NAD–MDH, peptidase A (Pep A), Pep B, Pep C, Pep D, phosphoglycerate kinase (PGK) phosphoglucomutase$_1$ (PGM_1), PGM_2, PGM_3, dimerictetrazolium oxidase (D–TO), glucose-6-phosphate dehydrogenase (G6PD), cytoplasmic glutamic oxaloacetic transaminase (C–GOT), red cell acid phosphatase (on starch gel), 6-phosphogluconate dehydrogenase (6PG D), NADH-diaphorase (Dia A) and NADPH-diaphorase (Dia B). Cellogel from chemetron was used as supporting medium, except for red cell acid phosphatase (Meera Khan 1971).

The family studies indicated that the electrophoretic variations found for Pep D, PGM_2, PGM_3 and D–TO were due to the existence of co-dominant alleles at the autosomal loci *Pep D*, PMG_2, PGM_3 and *D–TO*. PGM_2 and D–TO were predominantly red cell enzymes, PGM_3 variations were detectable in white cells, while Pep D variants were discernible in white cells as well as red cells, making them useful as markers in experimental bone marrow chimeras.

PGM_2, PGM_3 and *D–TO* were found to be di-allelic, whereas *Pep D* was tri-allelic, the gene frequency of the variant allele in each case being 0.010, 0.312, 0.054, 0.260 and 0.020, respectively. The rest of the systems exhibited no polymorphism. The Guelph group studied the following red cell enzymes on starch gel: LDH A, LDH B, Pep A, Pep B, Pep C, Pep D, Pep E, GPI and 6PGD. Only Pep D appeared to be polymorphic. Their results agreed with those of the Leyden group.

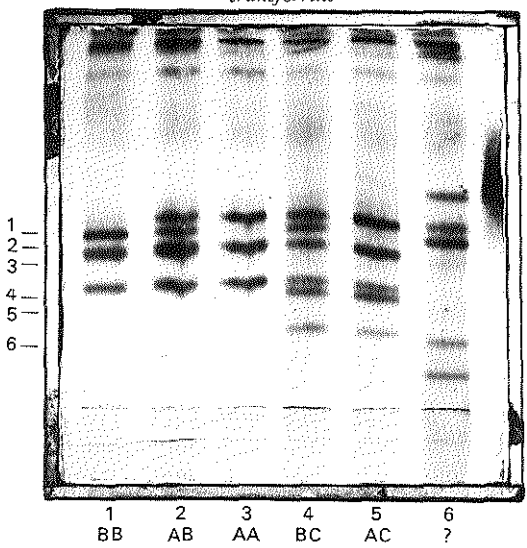
Serum Protein Polymorphisms

Transferrin (Tf): Braend (1966) and Stevens & Townsley (1970) have shown that the synthesis of canine serum transferrin is controlled by three autosomal co-dominant alleles which give rise to six phenotypes demonstrable by starch gel electrophoresis. An electrophoretic technique on thin agarose gel layers was used to test the workshop samples. This method is easier to perform and gives better results than starch gel electrophoresis.

Table 11 shows the electrophoretic patterns of six different unrelated dogs. Sample nos. 1 (bands 2–3–4) and 3 (bands 1–3–4) represent the homozygotes for TfB and TfA, respectively (phenotypes BB and AA). Sample no. 2 (bands 1–2–3–4) is the heterozygote TfA/TfB (phenotype AB). Sample nos. 4 (bands 1–2–3–4–5–6) and 5 (bands 1–3–4–5–6) have been classified as the heterozygotes for TfA and TfB and a third allele TfC. The homozygote for this allele has not been found in the group of dogs examined. The sixth sample does not fit in with the present classification. Appropriate breeding experiments are planned in order to obtain the homozygote TfC/TfC and to define the genotype of sample number 6. Table 12 gives the phenotype Tf distribution found in 86 littermates from five different mating types. The results are in agreement with expectations, based on a simple genetic system with three

Table 11

Agarose gel electrophoresis of canine transferrins



The number of the sample and the phenotype are indicated at the bottom of the photograph, near the origin. The bands of the first five types are numbered from 1 to 6 in order of decreasing mobility at the left side of the table.

co-dominant alleles, TfA, TfB and TfC. Stevens & Townsley observed a deficiency of homozygotes. In the present series of experiments the number of AA individuals from six AA × AB matings is lower than expected. The sample is, however, not large enough to allow definite conclusions. Table 13 shows the Tf phenotypes and gene frequencies in 94 unrelated dogs. The observed distribution of phenotypes is consistent with a series of alleles in Hardy-Weinberg equilibrium.

Table 13

Transferrin phenotypes and gene frequency in 94 unrelated Dutch mongrel dogs

Tf	Phenotypes		Gene frequency
	No. observed	No. expected	
AA	37	35[a]	A = 0.611
AB	39	43[a]	
BB	15	13[a]	B = 0.373
AC	2	2[a]	
BC	1	1[a]	C = 0.016
CC	0	(0.024)	
	94	94	

[a] Nearest integer.

Albumen (Alb): Townsley et al. (1972) have reported that albumen is polymorphic in dogs. A modification of their technique was used on serum samples from 31 unrelated Dutch mongrel dogs and 65 littermates from 11 matings representing five different mating types. Two alleles were defined. One gave rise to a fast moving band (A) and the other to a slower band

Table 12

Family studies of transferrin polymorphism

Mating types	Number of families	Phenotypes and number of offspring					
		AA	BB	CC	AB	AC	BC
AA × AA	1	6	–	–	–	–	–
AA × AB	6	13	–	–	23	–	–
AB × AB	3	9	5	–	14	–	–
AB × BB	3	–	6	–	6	–	–
AB × AC	1	2	–	–	–	2	–
Total	14	30	11	–	43	2	–

Table 14

Family studies of albumen polymorphism

Mating types	No. observed/expected phenotypes in littermates			
		Alb. A	Alb. AB	Alb. B
A × B	3	0/0	17/17	0/0
AB×AB	3	7/5	12/10	1/5
AB× B	3	0/0	3/6	9/6
B ×AB	1	0/0	6/5.5	5/5.5
A ×AB	1	1/2.5	4/2.5	0/0
Total	11	8/7.5	42/41.0	15/16.5

(B). The gene frequencies in the unrelated individuals were Alb A = 0.435 and Alb B = 0.564. Table 14 shows that the results of the family studies are compatible with expectations based on the genetic hypothesis of a bi-allelic system.

Linkage

DL–A segregation patterns in families were compared to the patterns of the following markers: the enzymes PGM_2, PGM_3, Pep D, D–TO; the erythrocyte antigens A, D and Tr; the lymphocytotoxic sera S 14060 and S 14113 (recognizing factors outside DL–A); digestive group system and the serum-proteins transferrin (Tf) and albumen (Alb). In no instance was there evidence for close linkage between DL–A and those markers. No striking similarities in the segregation patterns were observed for other combinations of these markers for which informative families were available. These studies will be continued with more advanced methods of analysis and results will be reported later.

Discussion

The evidence collected during this workshop clearly indicates the existence of two segregant series of multiple alleles in the DL–A region (see also Albert et al., abstract 5; Saison, abstract 6; and Vriesendorp et al. 1972a, 1973). These data *and* the significant delta values found between groups of the two segregant series, *and* the possible occurrence of cross reactivity between DL groups within the same series, indicate a striking similarity between DL–A, HL–A (Mattiuz et al. 1970) and RhL–A (Gabb et al. 1972, Balner et al. 1973).

The DL–A system appears to have a good potential for further studies in clinical histocompatibility. Important serological problems still remain to be solved. In human histocompatibility testing a higher serologic reproducibility has been found. Dog cells seem to be more liable to non specific death than human cells. Reproducibility rates will improve when optimal test conditions for dog cells are defined and when only those sera which show a high percentage of concordant results between duplicate tests are selected for further use. Some groups in the "second" segregant series, DL12 in particular, may be heterogenous, since two triplets and a deviation from Hardy-Weinberg equilibrium are observed in this series. A factor analysis of the clusters of positively correlated lymphocytotoxic sera with high population frequencies could possibly identify the different antibody populations within each cluster.

Some of the reasons for the high reaction frequencies of these reagents could be the use of unselected donor–recipient pairs and long immunization schedules. Currently the best available methods for the production of tissue typing reagents are a skin graft and/or lymphocyte injections between littermates which differ for one DL–A haplotype. As soon as a positive cross match between donor cells and recipient serum occurs, serum should be harvested (van der Does et al. 1972, abstract 8). It was shown that lymphocytotoxins in dog

sera are not necessarily directed against DL–A antigens. This has also been the case in human tissue typing (Dorf et al. 1972). For S 14113 (see Whitacre et al., abstract 7) and S 04003, immunizations were carried out between inbred animals. The donor-recipient combination which was still available for testing appeared to be DL–A identical (Schnappauf, unpublished observation). The prolonged immunization schedules could have been favorable for the development of lymphocytotoxins against factors other than DL–A. Shorter immunization periods in DL–A identicals have not produced that sort of antibody (Westbroek et al. 1972). In future studies of canine histocompatibility antigens, cytotoxicity patterns should be related to a genetic system, and the histocompatibility effect (major or minor) of such a system should be identified in the appropriate allografting experiments before definite conclusions can be reached. A beneficial influence of DL–A typing on allograft survival has been documented for bone marrow (Epstein et al. 1968) and many other organ allografts (for references see Westbroek et al. 1972) when littermates were used as donor–recipient pairs. Minor histocompatibility loci can now be analyzed in DL–A identical littermates.

The digestive tract groups and the new lymphocyte specificities (S 14060 and S 14113) are possible candidates for such a function. A study of the influence of the digestive tract groups on the occurrence of graft versus host (GVH) disease in DL–A identical dogs might be profitable, since an important part of the GVH reaction occurs in the digestive tract.

The possible existence of a separate MLC locus (or loci) in the dog shows that the dog might be closely analogous to mouse, rhesus monkey and man in the genetic fine structure of the major histocompatibility complex. It offers a model for proving the hypothesis for the recognition of "non self" and killing of allogenic cells (Eijsvoogel et al. 1972, Koch et al. 1972) by breeding "cross-over" dogs and obtaining pairs of individuals with incompatibility only for one DL-group or one DL-group + MLC determinant. Another interesting possibility is to attempt to breed animals which are homozygous for the major histocompatibility complex, including a DL–A and MLC region(s). Such a group of animals could provide a reference panel for the identification of "MLC determinants" in random individuals and for the determination of the influence of those factors on allografts of normal or malignant tissue. The availability of transplantable, spontaneously occurring tumors in the dog (TVT) will provide a good model for further studies of tumor-host relationships in an outbred species.

The canine erythrocyte antigens CEA 1 and 2 and possibly CEA 8 are of relevance in procedures involving multiple blood transfusions, since transfusion reactions have been reported to occur when incompatibilities existed for these antigens (Swisher & Young 1961). Sensitization or enhancement in future organ recipients by blood transfusions (Opelz et al. 1972) can now be investigated prospectively in the dog. The measurement of the immune response of different animals to red and white cell antigens might also lead to the discovery of Immune Response (IR) genes, a type of marker which is still unknown in the dog. The correlation between CEA 7 and digestive tract group A awaits further elucidation. An interesting possibility is that two *different* genetic loci exist, one for CEA 7 and one for digestive tract group A, which give rise to similar gene products. The other canine red cell antigens may be useful for genetic studies, such as linkage studies and paternity cases. The biological significance and the tissue distri-

bution of these antigens, except on erythrocytes, are as yet unknown. No relationships were found between canine erythrocyte antigens and DL groups. Rubinstein et al. (1968), however, have suggested that major histocompatibility antigens do occur on canine red cells. Experiments using a sensitive test and dog alloantisera of known DL specificity on canine red cells might resolve this interesting question.

Certain molecular analogies were noticed between the human and canine phosphoglucomutases (i.e. PGM_1, PGM_2 and PGM_3) suggesting a common evolutionary origin for the three forms of mutases in man and dog. Nevertheless, no measurable linkage was detected between the loci for DL-A and PGM_3 in male and female dogs. PGM_2, which is generally not polymorphic in man, was also not linked to DL-A. The serum protein and red and white cell enzyme markers can be very useful in liver and bone marrow grafting for tests checking the presence or absence of donor types. Multiple transferrin zones in homozygotes have been described in other animals besides the dog (Manwell & Ann Baker 1970). This problem could be further investigated by examining the chemical and physical properties of the protein moiety of purified Tf phenotypes. For the standardization of the Tf nomenclature, starch and agarose gel typing should be compared. This is in progress in the department of human genetics of the Leyden University.

The relationships between genetic markers (digestive tract group X, CEA 2, CEA 8 and DL 12) and the occurrence of autoimmune antibodies could well be fortuitous. More studies are necessary to determine if there is a significant biological relationship between these genetic markers and the occurrence of autoimmune antibodies. Some of the described polymorphisms might be examples of genetic systems with a heterozygote advantage (as the sickle cell trait in man). The worldwide distribution, short generation time and existing pedigrees of some dog breeds could provide a rich source of material for the study of whether genetic drift or selection is the force maintaining a given genetical polymorphism (Harris 1971). Earlier genetic studies in the dog have been concerned mainly with macroscopical anatomic structures, coat colors and disease states (Little 1957, Burns & Fraser 1966, Zweibaum, personal communication). This group of markers has not been investigated in the workshop. It can be expected that future studies will attempt to correlate the presence of disease states and the various immunogenetic markers in the dog. Besides the genetical interest of such investigations, they might produce useful marker genes for special dog diseases and thus enable the identification of heterozygotes for recessive disease genes. Phylogenetic relationships between canids and canine paternity cases are other areas which can be further investigated with the immunogenetic systems described.

Numerous other possible applications for the acquired insight into dog genetics were discussed during this workshop and symposium. It was felt that continued cooperation would be profitable. The general consensus was that there was a need for another workshop. J. W. Templeton agreed to assume the main responsibility for the organization of that second workshop. It will be held in November 1974 at the University of Oregon Medical School, Portland, Oregon, U.S.A.

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References

Andresen, E., Baker, L. N. & Rowe, K. E. (1963) The usefulness and limitation of two by two tables in distinguishing between allelism and non-allelism based on random population material. *Immunogenetic letter* **3**, 15–20.

Balner, H., d'Amaro, J., Dersjant, H., Vreeswijk, W. van & Toth, E. K. (1973) RhL–A. The major histocompatibility system of Rhesus monkeys. *Transplant. Proc.*, in press.

Bowdler, A. J., Bull, R. W., Slating, R. & Swisher, S. N. (1971) Tr: A canine red cell antigen related to the A antigen of human red cells. *Vox. Sang. (Basel)* **20**, 542–544.

Braend, M. (1966) Serum transferrin of dogs. *Proceeding of 10th Europ. Conf. Animal Blood Groups, Biochem. Polymorphism*, p. 319. van Dunk, Den Haag.

Burns, M. & Fraser, N. M. (1966) *Genetics of the Dog*, p. 17. Commonwealth Agricultural Bureaux, University Press, Aberdeen.

Cornelius, C. E. (1969) Animal models, a neglected medical resource. *New Engl. J. Med.* **281**, 934–944.

Does, J. A. van der, Rood, J. J. van, Walker, W. & Epstein, R. B. (1972) Consequent intra familial immunization for DL–A haplotyping in canines. *J. exp. Med.*, in press.

Dorf, M. E., Eguro, S. Y., Cabrera, G., Yunis, E. J. & Amos, D. B. (1972) Detection of cytotoxic not HL–A antisera. I. Relationship to antisera. *Vox Sang. (Basel)* **22**, 447.

Dungern & Hirschfeld, von (1910) Über gruppenspezifische Strukturen des Blutes. III. *Immunitätsf.*, 526.

Eijsvoogel, V. P., Du Bois, M. J. G. J., Melief, C. J. M., De Groot-Kooy, M. L., Koning, C., Rood, J. J. van, Leeuwen, A. van, Du Toit, E. & Schellekens, P. Th. A. (1972) Position of a locus determining mixed lymphocyte reactions (MLR), distinct from the known HL–A loci and its relation to cell mediated lympholysis (CML). *Histocompatibility Testing 1972*, in press. Munksgaard, Copenhagen.

Epstein, R. B., Storb, R., Ragde, H. & Thomas, E. D. (1968) Cytotoxic antisera for bone marrow grafting in littermate dogs. *Transplantation* **6**, 45.

Gabb, B. W., Piazza, A., D'Amaro, J. & Balner, H. (1972) Genetics of RhL–A system of Rhesus monkeys. *Transplant. Proc.* **4**, 11.

Harris, H. (1971) Polymorphism and protein evolution. The neutral mutation-random drift hypothesis. *J. med. Gen.* **8**, 444.

Koch, C. T., Hooff, J. P. van, Leeuwen, A. van, Tweel, J. G. van den, Frederiks, E., Steen, G. J. van der, Schippers, H. M. A. & Rood, J. J. van (1972) The relative importance of matching for the MLC versus the HL–A loci in organ transplantation. *Histocompatibility Testing 1972*, in press. Munksgaard, Copenhagen.

Little, C. C. (1957) *The Inheritance of Coat Colours in Dogs*. Comstock Publishing Associates, Ithaca, N.Y.

Manwell, C. & Baker, C. M. Ann (1970) Heterosis: Protein polymorphism and animal breeding. *Molecular Biology and the Origin of Species*. Sidgwick and Jackson, London.

Mattiuz, P. L., Ihde, D., Piazza, A., Cepellini, R. & Bodmer, W. F. (1970) New approaches to the population genetic and segregation analysis of the HL–A system. *Histocompatibility Testing 1970*. Munksgaard, Copenhagen.

Meera Khan, P. (1971) Enzyme electrophoresis on cellulose acetate gel. *Arch. Biochem. Biophys.* **145**, 470–487.

Opelz, G., Mickey, M. R. & Terasaki, P. I. (1972) Identification of unresponsive kidney-transplant recipients. *Lancet* **i**, 868.

Rood, J. J. van, & Leeuwen, A. van (1963) Leucocyte grouping, a method and its application. *J. clin. Invest.* **42**, 1382.

Rubinstein, P., Morgado, F., Blumenstock, D. A. & Ferrebee, J. W. (1968) Isohemagglutinins and histocompatibility in the dog. *Transplantation* **6**, 961–969.

Stevens, R. W. C. & Townsley, M. E. (1970) Canine serum transferrins. *J. Hered.* **61**, 71.

Swisher, S. N. & Young, L. E. (1961) The blood grouping systems of dogs. *Physiol. Rev.* **41**, 495.

Templeton, J. W. & Thomas, E. D. (1971)

Evidence for a major histocompatibility locus in the dog. *Transplantation* **11**, 429.

Townsley, M. E., Kraay, G. & Stevens, R. W. C. (1972) Polymorphism of canine serum albumin. Anim. Blood Grps. *Biochem. Genet.* **2**, 195–199.

Vriesendorp, H. M., Epstein, R. B., D'Amaro, J., Westbroek, D. L. & Rood, J. J. van (1972a) Polymorphism of the DL–A system. *Transplantation* **14**, 299.

Vriesendorp, H. M. (1972b) The occurrence of dog histocompatibility antigens in other canidae. *Genen. Phaenen,* **XV**, 73.

Vriesendorp, H. M., D'Amaro, J., Does, J. A. v. d., Westbroek, D. L. & Epstein, R. B. (1973) Analysis of the DL–A system in families and populations of healthy and diseased individuals. *Transplant. Proc.,* in press.

Westbroek, D. L., Silberbusch, J., Vriesendorp, H. M., Urk, H. van, Roemeling, H. W., Schönherr-Scholtes, Y. H. C. H. & Vries, M. J. de (1972) The influence of DL–A histocompatibility on the function and pathohistological changes in unmodified canine renal allografts. *Transplantation* **14**, 582.

Yasuda, N. & Kimura, M. (1968) A gene counting method of maximum likelihood for estimating gene frequencies in ABO and ABO-like systems. *Ann. hum. Genet.* **31**, 409.

Zweibaum, A., Oudea, P., Halpern, B. & Veyre, C. (1966) Presence in colic glandular cells of various mammalian species of an antigen, cross reacting with human blood group A substance. *Nature (Lond.)* **209**, 159.

Zweibaum, A. & Steudler, V. (1969) Présence chez le chien d'un système d'isoanticorps naturels spécifiques d'antigènes de groupe des sécrétions digestives. *Ann. Past.* **177**, 839–854.

CHAPTER 6

DISCUSSION AND CONCLUSIONS

6.1 INTRODUCTORY REMARKS

In the preceeding chapters a gradually developing insight into the major histocompatibility complex of the dog has been documented. Most of the details of this complicated genetic region still have to be explored. Three different genetic systems have so far been identified as constituents of the canine major histocompatibility complex, i.e. two systems or segregant series of multiple alleles controlling antigens, which can be recognized serologically and probably a third polymorphic system whose products control reactivity in the mixed lymphocyte cultures. This chapter will discuss the now available knowledge and some speculations will be made on future developments.

6.2 NOMENCLATURE

A uniform and simple nomenclature is a prerequisite for proper communications between investigators and between investigators and interested laymen. The use of complicated signs for the factors under study will damage this communication. An attempt will be made to circumvent this fallacy by explaining the nomenclature decisions made on DL-A in detail. It is used by most workers in the field and agrees with most of the prerequisites for a useful notation system. Swisher (153) listed these prerequisites.

Nomenclature symbols must be:

(1) unambiguous
(2) of simple format
(3) compatible with usual modes of communication, written, printed, spoken and machinetransmitted
(4) translatable into the major scientific languages of the world
(5) able to show, as far as possible, the historical development of the topic

(6) in a form, easily used in data processing systems
(7) made, so as to tolerate endless addition and deletion of symbols without necessitating alternative meanings for old symbols
(8) defined in some ancillary document, scientific paper or organized lexicon. This will enable one to put rather less information into the symbol itself, to make the symbol an unequivocal index into some type of glossary and to concentrate the information in the latter site.

The terms S.D. and L.D. have been introduced by Bach et al. (114) and have been explained in chapter 1 (1.3.1 and 1.3.2). In this way major histocompatibility structures can be divided into ones which are serologically defined (S.D.) and ones which control lymphocyte reactivity in mixed cultures (lymphocyte defined or L.D.). The exact role of either S.D. or L.D. determinants in the recognition of "foreign" (allogeneic) tissue is not known. An attractive hypothesis, put forward by Van Rood (96), and briefly discussed in chapter 1 (1.3.2) assumes that L.D. structures control the sensitization phase of the immune response of the recipient and S.D. structures the effector or destruction phase of this response. Extrapolating from the human data, which led to the formulation of this hypothesis one might anticipate that in dogs too, L.D. and S.D. structures would *both* play their separate role in the recognition and destruction of foreign tissues. Therefore, it would be unjustified to limit a common nomenclature for factors of the canine major histocompatibility complex DL-A to the serologically defined structures, although they are "historically speaking" older than the still insufficiently analysed lymphocyte defined structures. In accordance with the proposal of Elkins and Bach for mice (128), the term DL-A has been applied since the publication of the paper reprinted as chapter 5 in this thesis to all the three different systems so far identified (2 S.D. and 1 L.D. system). In this term D stands for dog, L for leucocyte; A is to indicate that this complex carries the *major* histocompatibility information and that it was the first genetic region to be discovered, which controls leucocyte antigens. Genetic systems, which will be identified on dog leucocytes in the future should thus be labelled DL-B, DL-C etc. Two nomenclature decisions had to be made for the three systems of the DL-A complex (1) name/symbol for locus, (2) name/symbol for alleles of different loci. The two S.D. loci have been labelled first series and second series, the L.D. locus has been called M.L.R. (mixed lymphocyte reactivity) locus. The alleles of the three systems can be given a discrete number from 1 to infinite. It has been proposed to reserve numbers 1-49 for S.D. alleles and 50-100 for L.D. alleles (Grosse - Wilde et al., manuscript in preperation). The prefix DL-A or DL can be used in front of the chosen number (see chapter 5). DL-A will be used when definite international agreement has been reached upon the recognition of a major histocompatibility structure. Before this has been achieved DL should be used for specificities which are recognized by many different laboratories, but whose analysis has not been completed yet. Locally discovered and recognized

specificities should be given a temporary local designation, until they are subjected to an international comparison test. For this purpose one could use for example the first letter of the home city of the investigator, followed by a number. If these nomenclature proposals are accepted, an international body of experts should be selected to evaluate periodically labels can be given to the emerging new factors (153). This committee should publish every two or three years an updated version of a list of all labels with a thorough description of the corresponding factors (see 8 of list of Swisher's prerequisites for a proper nomenclature). A successful cooperation of this sort has been achieved for human (e.g. 1), primate (116) and canine histocompatibility testing (see chapter 5). Every two years workshops are organized, in which the functions of the above mentioned "board of international experts" are fulfilled. The extensive treatment of nomenclature problems in this paragraph was inspired by the confusion that immunogenetical symbols usually engender and the hope that this unnecessary chaos can be circumvented in the field of dog histocompatibility by the consequent use of a sensible nomenclature.

6.3 POSITIONS OF SYSTEMS OF DL-A COMPLEX

Figure 6.1 gives the most propable relative positions of the three systems so far identified. The recombination frequency between 1st. and 2nd. S.D. series was estimated to be around 0.7% (chapter 4).
A rough estimate of the crossingover frequency between 2nd. S.D. series and M.L.R. can be made (around 1% or lower) on the basis of still unpublished results of Van den Tweel and coworkers. The evidence for the given sequence has been discussed in chapter 5 and originates from only two families with crossovers in the DL-A complex. Obviously more such families have to be studied before this concept can be definitely accepted.

Figure 6.1
The major histocompatibility complex of the dog.
Relative positions of systems.

Close linkage can be arbitrarily defined as the occurrence of different bits of genetical information on the same chromosome, which are separated by the event of crossing over with a frequency of less than 2%. One has, however, to keep in mind, that even between linked loci with this low recombination

frequency at least 250 genes must be located (121). The genes located between the three described DL-A systems could be related to the major histocompatibility complex. There is evidence available in other species that at least some of them are. In mice the Ss-Slp region, which is located between the two S.D. loci (see figure 6.3) was recently reported to be involved in the production of a substance of the complement system. The link of complement with immunological functions as graft rejection is evident.
In chapter one (1.3.3) other genetical systems have been discussed, which were found to be linked, to the M.H.C. in mice (72), guinea pigs (65), rats (46), rhesus monkeys (11) and possibly man (66), the socalled Immune response or Ir systems. In mice, no recombination has been observed so far between the MLR locus of the major histocompatibility complex of this species and the Ir-1 locus (115, 145). This lead to the hypothesis that M.L.R. determinants are products of Ir genes since both M.L.R. and Ir structures play probably a rôle in the recognition phase of the immune response (120). More insight into the mode of action of both structures is needed to allow a verification of this hypothesis.
This matter will be further discussed in the paragraph on the possible biological significance of the M.H.C. (6.5.3).

6.4 THE NUMBER OF S.D. AND L.D. LOCI IN A MAJOR HISTOCOMPATIBILITY COMPLEX

6.4.1 The number of S.D. loci

The existence of two S.D. loci in the M.L.C. has been challenged. Histocompatibility studies have generally presumed antibodies to be complex and antigens to be simple. However, it is a truism that when antisera are used to define polymorphic antigens, one starts with two unknowns, i.e. antisera and antigens. It was illustrated by Hirschfield in 1965 (136) that different conclusions can be reached in a genetic analysis of antigen-antibody relationship depending on the bias of the investigator. Thus a different picture of the number of S.D. parts of the major histocompatibility complex might be obtained, when other a priori assumptions are made (e.g. simple antibodies and complex antigens). Hirschfield identified two different starting points for analysis, opposite extremes of a whole scale of possibilities, neither of the two necessarily representing the true relationship of antigen and antibody. The first approach was labelled *simple-complex* since it assumes the antibodies to be simple and the antigens to be complex (consisting of more than one antigenic determinant). The second approach was given the name of *complex-simple*. Here one assumes the antibodies to be complex (one antibody can "crossreact" (see also 1.3.1) with two different antigens) and the antigens to be simple. Recently Klein (142) illustrated and reiterated this point for serological histocompatibility studies in

mice. The two different principles of analysis are shown in a modified version of a figure from Klein's paper (figure 6.2). In the figure a schematic illustration of the two models is given. In the first model two different antigens exist, which have a one-to-one relationship with the two antisera. It can be seen that in the second model antiserum A also reacts with antigens of cell Y, which fit antibody A, although less well than antigens from cell X. Different conclusions are reached in the two models on the presence of antigens and antibodies, as is evidenced by the last two columns in figure 6.2. In reality either of the two extremes, or a situation in between, in which some of the antigens and some of the antibodies are complex (a complex-complex model) might be prevelant. The important point here is that it cannot be decided, which of the alternative models is the most realistic, without detailed information on the biochemical structure of the antigens involved. Due to technical difficulties the exact chemical nature of major histocompatibility antigens is not yet known in any species (149). Genetic studies of major histocompatibility S.D. antigens have mainly used the complex-simple model with the exception of older studies in mice (152).

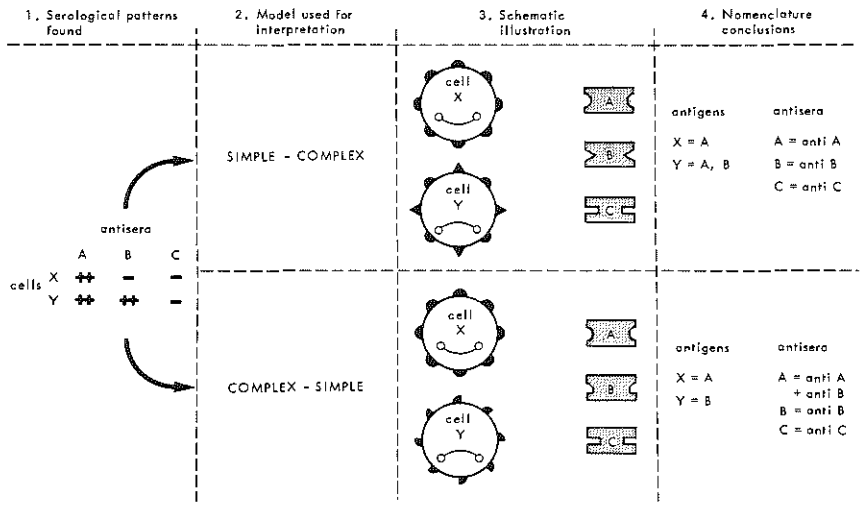

Figure 6.2
Two possible methods for a genetical analysis of serological results (after Hirschfield (134) and Klein (140)).

The preference for the complex-simple model for analysis is not completely correct, since it might not represent the best approximation of reality. It was demonstrated, however, to be an efficient and relatively simple way of describ-

ing and explaining commonly found reactivity patterns of antisera in men, mice, rhesus monkeys and dogs. Moreover biochemical (126) and immunofluorescent studies (146), have revealed the probable existence of at least two different molecules carrying S.D. specificities. However, several authors (113, 118, 124) have suggested that each S.D. locus, consists of subunits, each coding for a separate part of the major histocompatibility structure involved. Arguments in favour of this new hypothesis are: 1. it does not have the complex-simple bias, 2. sera which fail to support the two loci theory are commonly neglected as too complicated to explain. Both points can be solved theoretically with the assumption of two loci consisting each of several subunit. As already mentioned, the true and final picture of the detailed structure of the S.D. part of a M.H.S. will not be available until after a detailed biochemical characterisation of the antigens involved. Till that time the two loci concept will be a useful but still unverified model. Recently the probable existence of a third S.D. locus in man was reemphasized (141). In other mammals no more than two S.D. loci are still commonly thought to be present.

6.4.2 The number of L.D. loci

Not only the S.D. part of the M.H.C. might be more complex than the picture given in figure 6.1, since also the M.L.R. region might be more complicated. Eijsvoogel et al. (131) have obtained results in human mixed lymphocyte cultures, which they tentatively attributed to the effect of a second, weaker M.L.R. locus, in between the 1st. and 2nd. S.D. series of the human histocompatibility complex. Balner et al. (117) have in their investigations of the M.L.R. locus of rhesus monkeys described several results, which could not be explained by *one* M.L.R. locus alone. Festenstein and coworkers (132) have identified in mice a second genetic region, with an important influence on M.L.R. on a chromosome different from the one which carries the M.H.C. in this species. It can thus be concluded from these reports, that in several species more than one genetic system influences reactivity in mixed lymphocyte cultures. However, in all species so far investigated there is at least *one* locus with a major influence which appears to be linked to the S.D. part of the M.H.S. (see also next paragraph). Also in dogs data have recently become available indicating that more than one M.L.R. system does exist in this species (Van den Tweel, personal communication). Summarizing 6.4 it can be said that neither the definitive number of S.D. loci, neither the number of L.D. loci has been found.

6.5 FUNCTION OF MAJOR HISTOCOMPATIBILITY COMPLEX (M.L.C.)

6.5.1 Similarities of mammalian M.H.C.'s and occurrence of M.H.C.'s in non mammalian species

Notwithstanding the still unsolved problems of the finer structure of M.H.C.'s in all species, a study of the literature reveals that mammals have very similar M.H.C.'s. This phenomenon has already been mentioned in this thesis (1.3.3). In

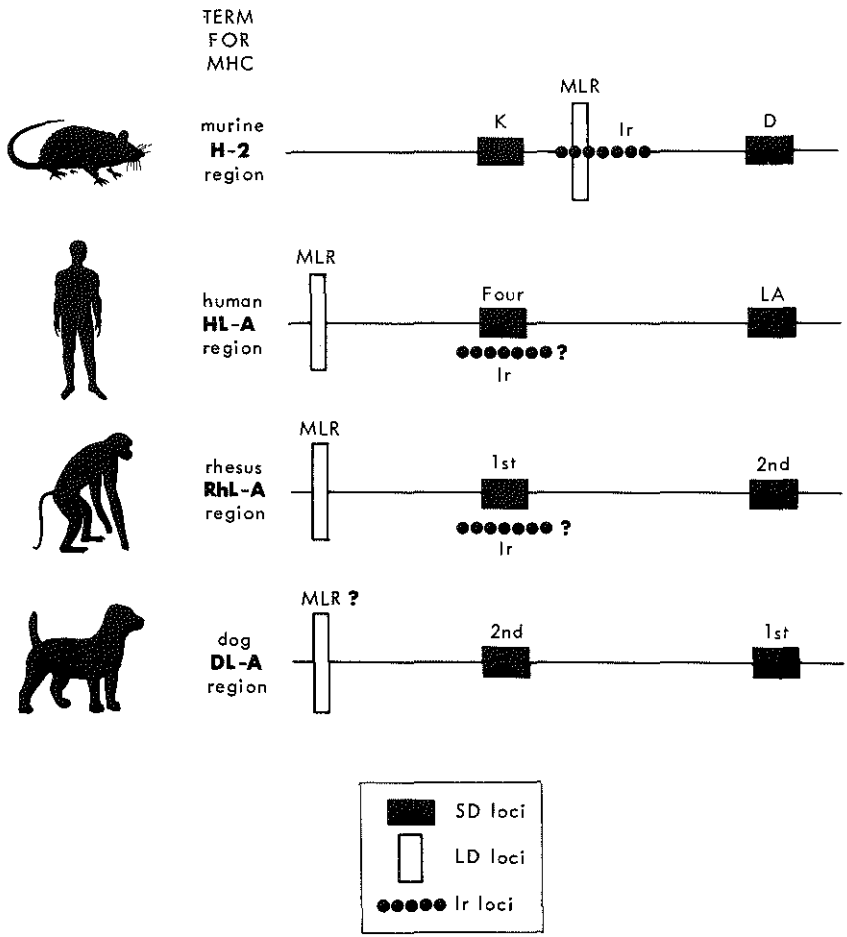

Figure 6.3
Major histocompatibility complex of four different mammalian species.
Terms L.D., S.D. and Ir are explained in text.

figure 6.3, the data are depicted for the major histocompatibility complex of mice (142), men (32), rhesus monkeys (10, 11), and dogs (this thesis). To obtain the full story behind the figure for each species, the reader is advised to consult the given references. All species appear to have at least two S.D. loci and one M.L.R. locus and in those species in which Ir genes have been defined, at least some of the Ir systems appear to be linked with the M.H.C. Exact mapping data for Ir genes are not yet available for man and rhesus monkey, which is the reason for the question marks in the figure. Their presence has not been ascertained yet in dogs. Clearcut evidence for serological and/or biochemical similarities in M.H.C. structures of different species has not been found, with the exception of men and chimpanzees (9, 12, 30) and foxes and dogs (chapter 5). Vague indications for serological similarities of S.D. antigens of man and mouse have been found (125). Less information is available on M.H.C.'s in other mammalian species, not included in figure 6.3 (as rats, rabbits and guinea pigs). One should, however, keep in mind, that M.H.C.'s not only occur in mammals. Chickens also appear to posses one (137) and recently evidence has been found suggesting the existence of a M.L.R. locus in the amphibian xenopus laevis (148).

6.5.2 Function in transplantation immunology

Different functions for L.D. and S.D. genes

The important rôle of the M.H.C. in tissue transplantation has been clearly established in all the mammalian species investigated so far.
The earlier mentioned hypothesis, which can be found in the papers of Van Rood (96) and Eijsvoogel et al. (131), supposes that in transplant rejection L.D. and S.D. structures have different functions, which are related to the phase of the recipient's immune response. In phase one, (sensitisation phase) the recipient would recognize and be sensitized for the allogeneic material through the difference between the L.D. products of its own and those of the donor. In phase two (destruction phase) the recipient's immune system attacks the donor cells. Antigens controlled by the two S.D. loci serve as targets for this attack. Antigens of different S.D. series might not be of the same importance as targets. A difference in effect has been observed in mice (135), man (53, 131) and possibly dogs (Westbroek et al. unpublished observation). K, Four and second series antigens appear to have a greater influence on graft survival than D, LA and first series antigens. Grafts which differ for K, four or second series antigens with their recipients survive shorter than those which differ only for the D, LA or first series antigens. In man this effect was most clearly demonstrated in allograft recipients, which had received bloodtransfusions and subsequently produced antibodies against leucocyte antigens before kidney transplantation (53). This difference in importance of antigens of the two S.D. series cannot be

explained yet. It might be due to a difference in density in the cell membrane (a quantitative difference). A qualitative difference between antigens of different series is another possible explanation.
Not only graft survival, but also graft histology and function appear to be influenced by the differences between donor and recipient for the M.H.C., Westbroek et al. (155) have shown that kidney grafts exchanged between DL-A identical sibs show biochemical and histological patterns of rejection different from those observed in grafts exchanged between DL-A *non* identical sibs. Another property of the M.H.C. is its influence on the effect of an immunosuppressive agent. For example de Gruyl et al. (134) have documented that a particular batch of ALS had a greater influence on pancreatic allograft survival in partially matched related DL-A donor-recipient pairs than in DL-A totally mismatched, unrelated donor-recipient pairs. The same has been shown in small bowel and kidney allografts (Rothengatter et al. (150), Obertop unpublished observation).
Results in small rodents have shown that the phenomena of enhancement and tolerance (for a definition of these terms see (138) and (151) respectively) are more easily produced in host-donor combinations, which differ for minor histocompatibility antigens only. Preliminary results of Jeekel et al. (139) suggest that the same holds for outbred animals e.g. dogs. Their studies, which are still in progress, seem to indicate that it is necessary for the evaluation and predictable production of enhancement in dogs to determine and minimize the M.H.C. differences between donor and recipient.
In summary, it has become clear that the effect of the M.H.C. similarities or differences can be noted not only in graft survival, but in many ways. An exact definition of the rôle of the different systems of the M.H.C. in transplantation biology is still lacking. Some indications for differences in the rôle of S.D. and L.D. structures in transplant rejection have been found.

6.5.3 Function in biology

The search for a biological function of M.H.C.'s has been discussed in chapter one. It was mentioned there that no precise function has yet been identified. Suggestive evidence for an "immunological" rôle in biology has, however, been found. In an earlier paragraph (6.5.1) and figure 6.3 it was shown that mammalian M.H.C.'s resemble each other. One of the traits in common is the existence of close linkage between at least three systems with related functions. It is still unknown (123), whether the observed linkage between Ir genes and the major histocompatibility complex in several mammalian species is an indication of some functional relationship between the two. Several hypotheses on the biological function of Ir genes and histocompatibility assume that this is in fact the case (120, 122, 140). Experiments of Green et al. (133) are compatible with but

do not give conclusive evidence that there is a relationship between Ir gene products and S.D. major histocompatibility antigens, at least in vitro. Ohno (147) has, however, stressed that linkage per se does not indicate a functional relationship, since some functional related genes are linked, while others are not. More data are needed to improve our understanding of the relationships between the cluster of closely linked genetic systems which have been discussed in this thesis. The fact that important diseases have been shown to be related to the major histocompatibility complex and/or Ir-systems (see 1.3.3), is the main rationale for the many extensive studies, which are being done now in that direction.

6.6 DISCREPANCIES BETWEEN RESULTS OF HISTOCOMPATIBILITY TESTING IN RELATED COMPARED TO UNRELATED DONOR RECIPIENT PAIRS

In man (53) and rhesus monkey (127) it was found that typing and matching donor and recipient for S.D. antigens was not as effective in prolonging graft survival, when donor and recipient were unrelated, compared to the clear cut improvement in survival of S.D. matched grafts of related donors. In Beagles transplantation experiments have been performed, by Westbroek and his colleagues, in related and unrelated donor recipient combinations, which were typed for serological DL-A antigens (154, and Westbroek et al. unpublished observations). Figure 6.4 shows the survival times of their different experimental groups. It is clear from the figure that in this model typing *does* have a pronounced effect on survival times of organs from *unrelated*, DL-A identical, donor recipient pairs. Two questions arise on the basis of the above mentioned data

(1) What is the reason for the difference between related and unrelated donors?
(2) Why do dogs appear to do better than primates, when organs from unrelated but S.D. compatible donors are used for grafting?

Question (1) has been discussed in chapter one. Briefly the best available answer at the moment is that besides S.D. determinants, L.D. determinants and, perhaps other genetically controlled structures (Ir gene products?) of a M.H.C., are also important for graft survival.

In *related* donor recipient pairs, identity for S.D. antigens will generally *also* mean identity for the other important M.H.C. parts, since crossing over in the M.H.C. region is rather infrequent. In *unrelated* donor recipient identity for S.D. antigens will *not* necessarily imply identity for the other parts of the M.H.C. region. The occurrence of linkage disequilibrium between parts of the M.H.C. will nevertheless cause a higher frequency of identity for the total of M.H.C. information in unrelated donor recipient pairs, which are S.D. identical than in pairs which are not. For example in humans and rhesus monkeys approximately

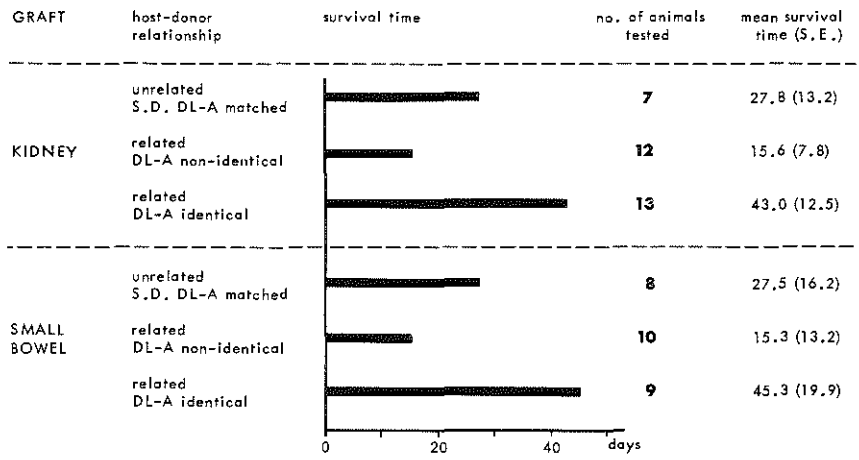

Figure 6.4

Survival times of DL-A S.D. compatible grafts from unrelated donors, compared to survival times of DL-A identical grafts from related donors.

10% of S.D. identical unrelated donor recipients pairs have been found to produce low or even negative responses in mixed lymphocyte cultures, while in S.D. nonidentical combinations only a very occasional low or negative response is found (131, Balner, unpublished observation). This would explain why sometimes an improved survival can be found with the use of unrelated donors typed for S.D. antigens, since individuals with identical S.D. structures would have a higher chance of being identical for L.D. structures too. Although this explanation is attractive, it has yet to be corroborated by allografting in the following experimental groups of unrelated donor recipient pairs.

a. L.D. identical, S.D. identical combinations.
b. L.D. identical, S.D. non-identical combinations.
c. L.D. non-identical, S.D. identical combinations.

A comparison of the survival times in these groups should reveal the influence of L.D. matching on graft survival and whether other structures different from L.D. and S.D. may also play a rôle. The first data related to this subject have come from Koch et al. (143). They seem to indicate that M.L.C. responses and thus L.D. determinants are important. The possibility that still other structures of the M.H.C. play an additional role in the determination of graft survival cannot be excluded.

For question (2) (the reason for a greater efficiency of S.D. typing in unrelated canine donor recipient pairs, when compared to primate donor recipient pairs) the three, most evident, possible answers will be discussed here. A first, simple, explanation would be a limited amount of genetic variation in the Beagles used

by Westbroek et al., due to inbreeding. They were unrelated as far as their pedigrees go (2-3 generations), but could have had common ancestors before. Unrelated dogs would in fact be related, and thus explain the discrepancy between dogs and primates. The explanation does not hold for the data reported by Epstein et al. (129) on dog bone marrow grafting, in which donor and recipient were in almost all cases of different breeds. A beneficial effect of an at that time still limited S.D. typing and matching could nevertheless be demonstrated.

A second explanation could be a lower degree of polymorphism of the M.L.C. locus in dogs than in primates. This would increase the possibility of two unrelated dogs having S.D. as well as L.D. structures of the M.H.C. identical, when only the S.D. antigens are being matched. When identify for S.D. *and* L.D. would be better for graft survival than identity for S.D. alone this would supply the answer asked for. Data reported by Meera Khan et al. (144) would support the second explanation. They described that only 4 out of 24 different enzymes were polymorphic in dogs. In other species commonly a higher percentage of polymorphic enzyme systems have been found. When a lower number of genetic systems in dogs is polymorphic or when less alleles are present in an average polymorphic genetic system, this could be explained by the assumption of a so-called bottle neck, or founder effect (see 21). Periods might have existed in dog history, in which the number of mating individuals per area was limited. This would have caused a loss of genetic variability. This holds a fortiori for a special dog breed (such as the Beagle), where the number of mating animals is limited by the desire of their owners to breed champions. For this purpose only a limited number of "top quality" sires and bitches are allowed to breed. Arguments against this limited amount of genetic variation in dog are that Beagles were found to show marked polymorphism for S.D. determinants of the DL-A complex (see chapter 4) and were in Hardy Weinberg equilibrium for these antigens (chapters 3 and 4). This also excludes recent severe inbreeding as a source of limited genetic variation, but does of course not supply an answer for the possible loss of variability through the presence of a bottle neck or founder effect for genetic systems other then the S.D. series (as M.L.R.). Another argument against a limited amount or loss of genetic variation in dogs is the well-known great diversity of this species for mainly polygenically determined traits such as body size, coat colour, coat texture etc., although some of the more extreme variations have undoubtedly been produced artificially by the selection of the dedicated dog breeders for the traits in question. Ultimately the determination of the frequency of M.L.C. negative combinations in unrelated dogs will show whether there is in fact a low degree of polymorphism for this genetic information in dogs. Preliminary experiments of Van den Tweel (personal communication) indicate that the precentage dog pairs, which is non reactive in unilateral M.L.C.'s is approximately 5%. This shows that the M.L.C. system

would be as polymorphic as the S.D. systems of the canine major histocompatibility complex. This amount of polymorphy would be sufficient to cause rejection of the majority of S.D. matched organs from unrelated donors, if L.D. structures are indeed important in that respect.
The third and at the present most likely explanation for the found differences between dogs and primates is suggested by still other results of canine M.L.C.'s from Van den Tweel and coworkers. They found a very high percentage of M.L.C. negative donor recipient pairs in unrelated S.D. identical pairs of mongrel dogs i.e. ± 50%, which is much higher than the percentage found in primates in the equivalent situation, i.e. ± 10%. This can be explained by a higher degree of linkage disequilibrium between L.D. and S.D. structures of the canine major histocompatibility complex. This outspoken linkage disequilibrium might for a yet undetermined part be caused by the earlier mentioned founder effect.
A high percentage of unrelated individuals which are identical for S.D. as well as L.D. parts of the M.H.C. in dogs is very useful for further studies, since these combinations are difficult to come by in other species. Moreover the availability of several dogs homozygous for L.D. alleles ((29) and Grosse-Wilde, personal communication) will enable a relatively easy identification of donor recipient pairs, which are L.D. identical but S.D. non identical. Thus all the host-donor combinations relevant for a better evaluation of the influence of matching on graft survival in unrelated donor-recipient pairs can be found in the dog.

6.7 SUGGESTIONS FOR FURTHER EXPERIMENTS

The experimental usefulness of dogs needs hardly any explanation. Surgeons commonly use and will use dogs in their laboratories. The size, temperament and availability of dogs are important reasons for this preference.
A further exploration of the M.H.C. of the dog is therefore warranted. Ideally the knowledge of DL-A should reach a level comparable to the level of HL-A knowledge, so that feasibility studies can be performed in dogs, instead of in humans. This can be exemplified by the unwarranted application of enhancement in human kidney transplantations (119), in which recipients have received anti donor antisera. Optimal conditions for the induction of tolerance or enhancement in outbred animals still need to be defined. Studies in e.g. dogs or other non-human outbred species should have preceded the clinical studies mentioned. The preliminary results of Jeekel et al. (139) have underlined this need.
Not only the transplant surgeon will benefit from a further exploration of the DL-A complex. The factors determining susceptibility to common diseases might be better defined, if further studies do indeed show a relationship between Ir genes and the DL-A complex. Further genetic studies of DL-A would benefit tremendously from an exact biochemical determination of its gene products.

This will be a formidable task, in the light of the great difficulties encountered in similar studies in other species. A list of other problems which need to be solved soon for further progress in dog histocompatibility studies can be constructed from the preceeding chapters.

This list includes

(1) a closer look at canine L.D. structures. Suggestions for a population analysis of these factors have been given.
(2) investigations into the possible presence and linkage relationships of Ir genes in dogs. Recently such studies have been initiated.
(3) allotypes for immunoglobulins have been defined in most laboratory animals but are not yet known in dogs.

The studies described in this thesis were started in the hope that eventually the dog could serve as an experimental model to test new concepts in immunobiology in outbred animals before application in humans. Clearly not enough has been achieved fully to attain this goal. A better definition of the experimental subject still needs to be achieved. Future studies and a continuation of the internal cooperation, which has been initiated in this field (see chapter 5) will almost certainly achieve this lofty ambition. For the purpose of experimental surgeons an already fair degree of definition of the experimental subject has been reached. In the light of the many advantages of dogs it is to be wished, that further progress in dog immunogenetics will provide an experimental animal with an even greater applicability.

6.8 References

References with numbers under 113 are listed in paragraph 1.4 of chapter 1.

113. Amos. B. 1971. Genetic control of the human HL-A histocompatibility system: alternatives to the two sublocus hypothesis. Transplantation Proceedings 3, 71.
114. Bach, F.H., Widmer, M.B., Bach, M.L. and Klein, J. 1972. Serologically defined and lymphocyte defined components of the major histocompatibility complex. J. Exp. Med. 136, 1430.
115. Bach, M.L., Widmer, M.B., Bach, F.H. and Klein, J. 1973. Mixed leucocyte cultures and immune response region disparity. Transplantation Proceedings 5, 369.
116. Balner, H. and Rood, J.J. van, (eds.). Transplantation Genetics of Primates. 1972. Transplantation Proceedings 4, 1.
117. Balner, H. and Toth, E.K. 1973. The histocompatibility complex of Rhesus Monkeys II. A major locus controlling reactivity in mixed lymphocyte cultures. Tissue Antigens (In press).
118. Batchelor, J.R. and Selwood, N.H. 1970. *In*: Terasaki, P.I. (ed.). HL-A subloci: Are they valid? . Histocompatibility Testing 1970, p. 243. Munksgaard, Copenhagen.
119. Batchelor, J.R., Ellis, F., French, M.E., Bervick, M., Cameron, J.S. and Ogg, C.S. 1970. Immunological enhancement of human kidney graft. Lancet ii, 1007.
120. Benacerraf, B. and McDevitt, H.O. 1972. Histocompatibility linked immune response genes. Science 175, 273.
121. Bodmer, W.F., Bodmer, J.G. and Tripp, M.V. 1970. Recombination between the LA and 4 loci of the HL-A system *In*: Terasaki, P.I. (ed.). Histocompatibility Testing 1970, p. 187. Munksgaard, Copenhagen.

122. Bodmer, W.F. 1972. Evolutionary significance of the HL-A system. Nature 237, 139.
123. Cohn, M. 1972. *In*: McDevitt, H.O. and Landry, M. (eds.). Genetic control of immune responsiveness. Relationship to disease susceptibility, p. 427. Academic Press, New York.
124. Dausset, J. 1971. The genetics of transplantation antigens. Transplantation Proceedings 3, 8.
125. David, C.S., Klein, J. and Shreffler, D.C. 1973. Serologic homology between H-2, and HL-A system. Transplantation Proceedings 5, 461.
126. Davies, D.A.L. 1969. The Molecular individuality of different mouse H-2 histocompatibility specificities determined by single genotypes. Transplantation 8, 51.
127. Dicke, H.W., Marquet, R.L. Heystek, G.A., and Balner, H. 1971. Effect of immunosuppressive treatment and leucocyte antigen matching on kidney allografts in Rhesus monkeys. Transplantation Proceedings 3, 484.
128. Elkins, W.L. and Bach, F.H. 1973. Danger of doctrinal tyranny in the terminology of H-2. Cellular Immunology 7, 522.
129. Epstein, R.B., Storb, R. and Thomas, E.D. 1971. Relation of canine histocompatibility testing to marrow grafting. Transplantation Proceedings 3, 161.
130. Eijsvoogel, V.P., Schellekens, P.T.A., Breur-Vriesendorp, B., Koning, L., Koch, C., Leeuwen, A. van, and Rood, J.J. van 1971. Mixed lymphocyte cultures and HL-A. Transplantation Proceedings 3, 85.
131. Eijsvoogel, V.P. du Bois, R., Melief, C.J.M., Zeylemaker, Z., Raat-Koning, L. and de Groot-Kooy, L. 1973. Lymphocyte activiation and destruction in vitro in relation to MLC and HL-A. Transplantation Proceedings 5, 415.
132. Festenstein, H., Abbasi, K., Sachs, J.A. and Oliver, R.T.D. 1972. Serologically undectable immune response in transplantations. Transplantation Proceedings 4, 219.
133. Green, Shevach and Paul 1972. *In*: McDevitt, H.O. and Landry, M. (eds.). Genetic control of immune responsiveness. Relationship to disease susceptibility, p. 19. Academic Press, New York.
134. Gruyl, J. de, Westbroek, D.L., Dijkhuis, C.M., Vriesendorp, H.M., MacDicken, I., Hlion Gerritsen, W., Verschoor, L., Hulsmans, H.A.M. and Hörchner, P. 1973. Influence of DL-A matching, ALS and 24 hour preservation on isolated pancreas allograft survival. Transplantation Proceedings 5, 755.
135. Hilgert, I. and Nouza, K. 1970. Strength of H-2 and non-H-2 antigens as compared in ALS suppressed recipeints. Folia Biol (Praha) 16, 369.
136. Hirschfield, J. 1965. Serologic codes, interpretation of immunogenetic systems. Science 148, 968.
137. Ivany, P. 1970. *In*: Current topics in microbiology and immunology 53, p. 8. Springer, Heidelberg.
138. Jeekel, J. 1971. Immunological enhancement of skin allo- and heterografts. Thesis, Erasmus University Rotterdam.
139. Jeekel, J., Obertop, H., Bauer, F.L.R., Vriesendorp, H.M. and Westbroek, D.L. 1973. Attempts to induce enhancement of canine kidney allografts. Tissue Antigens 3, 171.
140. Jerne, N.K. 1971. The somatic generation of immune recognition. Eur. J. Immunol, 1.1.
141. Kissmeyer-Nielsen, F., Svejgaard, A. and Thorsby, A. 1971. Possibility of a third sublocus. Transplantation Proceedings 3, 81.
142. Klein, J. 1973. The H-2 system. Past and Present. Transplantation Proceedings 5, 11.
143. Koch, C.T., Hooff, J.P. van, Leeuwen, A. van, Tweel, T.G. van den, Frederiks, E., Steen, G.J. van der, Schippers, H.M.A. and Rood, J.J. van 1973. The relative importance of matching for the MLC versus the HL-A loci in organ transplantation. *In*:

Dausset, J. and Colombani, J. (eds.). Histocompatibility Testing 1972, p. 521. Munksgaard, Copenhagen.
144. Meera Khan, P., Los, W.R.T., Does, J.A. van der and Epstein, R.B. 1973. Isoenzyme markers in dog blood cells. Transplantation **15**, 624.
145. Meo, T., Vives, J., Miggiano, V, and Schreffler, O. 1973. Major rôle for the Ir-1 region of the mouse H-2 complex in the mixed leucocyte reaction. Transplantation Proceedings **5**, 377.
146. Neauport-Sautes, C., Silvestre, D., Kourilsky, F.M. and Dausset, J. 1973. Independence of HL-A antigens from the first and second locus at the cell surface. *In*: Dausset, J. and Colombani, J. (eds.). Histocompatibility Testing 1972, p. 539. Munksgaard, Copenhagen.
147. Ohno, S. 1970. Evolution by Gene Duplication, p. 42. Springer Verlag, Berlin.
148. Pasquier, L. du 1972. Paper given at Conference on Cellular Immunity in Mice at the London Hospital and Medical College, London.
149. Reisfeld, R.A., Pellegrino, M.A., Ferrone, S. and Kahan, B.D. 1973. Chemical and Molecular nature of HL-A antigens. Transplantation Proceedings **5**, 447.
150. Rothengatter, C., Westbroek, D.L., Vriesendorp, H.M. and Vries, M.J. de 1971. *In*: Benmark, S. and Olsen, A. (eds.). Abstracts of 6th Congress European Society for Experimental Surgery, p. 65. Rahms Bokkeryy, A.B. Lund, Sweden.
151. Russell, P.S. and Monaco, A.P. 1964. Biology of tissue transplantation, p. 56. J.A. Churchill, London.
152. Schreffler, D.C. 1970. Immunogenetics of the mouse H-2 system. *In*: Aminoff, D. (ed.). Blood and Tissue Antigens, p. 85. Academic Press, New York.
153. Swisher, S.N. 1963. Biochemical notation systems. J. of the American Med. Association **185**, 21.
154. Westbroek, D.L., Rothengatter, C., Vriesendorp, H.M., Rood, J.J. van, Willighagen, R.G.J. and Vries, M.J. de 1971. Histocompatibility and allograft rejection in canine small bowel transplants. Evidence for the existence of a major histocompatibility locus in the dog. Transplantation Proceedings **3**, 157.
155. Westbroek, D.L., Silberbusch, J., Vriesendorp, H.M., Urk, H. van, Roemeling, H.W., Schönherr-Scholtes, Y.H.C.H. and Vries, M.J. de 1972. The influence of DL-A histocompatibility on the function and pathohistological changes in unmodified canine renal allografts. Transplantation. **14**, 582.

APPENDIX

One stage micro-cytotoxicity test.

This test is a variation of the methods described by Kissmeyer-Nielsen and Kjerbye 1967, Boyum 1968, van Rood et al. 1970.

Cell separation.

1. 5cc of blood is taken in heparine from the vena cephalica anterior or the vena jugularis. The heparine should be free of toxicity for lymphocytes (e.g. 2 drops of thromboliquine of Organon Oss, The Netherlands, the equivalent of approximately 500 I.U.).
2. Add a trace of carnonyl iron powder (General Aniline and Film Corporation, 140 West 51 St., New York N.Y. 10020, 341 product code 1-1-63763).
3. Swirl for 10′ at 37° C in waterbath.
4. Add 5% dextran (M.W. 200.000; Poviet, Amsterdam) in saline. Let mixture sediment for 30′ in upright position at 37°C in waterbath.
5. Remove supernatant. Layer ± 1.5 ml of supernatant over 1 ml of ficoll-isopaque mixture. This should be done carefully without disturbing the interphase. Tubes should be about 11 mm in diameter and about 80 mm long. Preparation of Ficoli-Isopaque solution: Isopaque 440 (440 mg I/ml. pro injection; Nyegaard 10 AS, Oslo, Norway). Ficoll Mw light scattering ca. 400.000: Pharmacia Uppsala, Uppsala, Sweden. The final mixture consists of 10 parts 33.9 percent isopaque and 24 parts 9 percent ficoll. Final density should be 1.077 g/ml.
6. Spin 15′ at 1000 g. Remove lymphocytes from interphase, take care to remove as little Ficoll-Isopaque as possible. Wash twice in Hanks BSS. Spin cells down at 400 g for 5′.
7. Add 4 drops of undiluted fresh whole rabbit serum. Count number of lymphocytes and adjust to approximately 6000/mm^3 by addition of more rabbit serum. The rabbit serum should be screened for "naturally occurring"

antidog lymphocytotoxins and complement activity. This can be done by testing lymphocyte samples of 5 different dogs with rabbit serum and with inactivated dog alloantisera, which are known to react positively and negatively with the cell samples to be studied. Only sera from rabbits with no "natural occurring" antibody activity against dog lymphocytes and a with a good complement activity should be pooled and used as complement source.

Test.

1. Trays (Coates Logos, Rabekk gt. 3, Moss, Norway) are filled with paraffin. A nontoxic paraffin should be used (e.g. Medinol 195). 1 μl of the antisera are injected into the wells, under the Medinol oil with a Hamilton microsyringe. Before use antisera have to centrifuged at a high speed to remove the debri in the serum which will interfere with a proper reading of the test.
2. Add 1 μl of lymphocyte suspension. Mix serum and cell suspension by gentle shaking.
   Incubate for 30′ at 37° C.
3. Mix 2% trypan blue in distilled water with an equal volume of 1,8% NaCl. Centrifuge mixture to remove particulate matter at 2000 g for 10′ Add 0.5 μl of the dye to the cell serum mixture.
4. Incubate for 30′ at room temperature or for 10′ at 37° C. After incubation place glass coverslip (Chance Microscope Cover Glasses 28 x 53 mm) over the droplets and press gently to spread droplets out.
5. The test is read with an inverted microscope and scored by visual estimation of percent of cells, which have taken up the dye.

score	% dye uptake
–	0
(+)	0 -10%
+	10-25%
++	25-50%
+++	50-75%
++++	75-100%

Storage of trays prefilled with antisera at –20° C.
Storage of whole blood for 2-3 days at 4° C before processing for cellsuspensions is possible.

Boyum A. (1968) Separation of leucocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest, 21 Suppl. 97.

Kissmeyer-Nielsen F. and Kjerbey F.E. (1967) Lymphocytotoxic Microtechnique purification of lymphocytes by flotation. Histocompatibility Testing 1967, pp. 381-387 Munksgaard, Copenhagen.

van Rood J.J., van Leeuwen A. and Zweerus R. (1970) The 4A and 4B antigens: Do they or don't they. Histocompatibility Testing 1970, pp. 93-103, Munksgaard, Copenhagen.

SUMMARY

In chapter 1 an introduction into the field of histocompatibility is given. A historical survey of important landmarks in two different lines of research is used for this purpose, i.e. 1. experimental transplantation of malignant cells and 2. the determination of differences between individuals by serological methods. The second part of chapter 1 is devoted to the description of some of the current topics in histocompatibility research. In sequence are discussed the demonstration of the existence of major histocompatibility complex, cellular methods of measuring histocompatibility and the biological significance of histocompatibility. Chapters 2, 3, 4 and 5 have been published previously as separate scientific communications in which shorter descriptions of results are given, than in the commonly more elaborate thesis, not based on earlier published results. For that reason chapter 1 has also been written as a guide to these latter chapters.

Chapter 2 describes the production and evaluation of serological tissue typing reagents in dogs. The existence of a major histocompatibility system in dogs is demonstrated. Later this system will be promoted to a complex, since it appears to be more complicated than was primarily assumed (see chapters 5 and 6).

In chapter 3 the first serologically defined antigens of the DL-A complex are described. Evidence for the existence of at least two series of multiple alleles controlling DL-A antigens is presented.

Chapter 4 gives more information on the DL-groups of chapter 3 and adds several groups to those already known. Recombination between the two series is reported. Associations between DL-groups of different series in a dog population are documented, suggesting a fair degree of linkage disequilibrium between some alleles of the two series. An association between DL-7 and the disease leukosis is found. Further studies are needed to corroborate this. A survey of the survival times of different organs from donors matched for the major histocompatibility complex is given.

Chapter 5 is the report of the first international Workshop of Canine Immunogenetics which was held in Rotterdam at the Medical Faculty of the Erasmus

University of Rotterdam, from November 5 - 17 in 1972. Previously described serologically defined DL-groups have been confirmed by other workers in the field. One new DL-group has been found on this occasion. The Rotterdam-Leiden nomenclature has been accepted for further use until the next workshop. The conclusion has been reached that probably a third genetic system is present in the DL-A region, which controls reactivity in mixed lymphocyte cultures. Two serologically defined specificities, which occur on lymphocytes, but which are different from DL-A antigens, were identified. They are probably controlled by alleles of the same genetic system. Other markers such as red cell antigens, digestive tract groups, red and white cell enzyme polymorphisms and serum protein polymorphisms have been investigated during the workshop. No evidence for linkage groups in dogs has been obtained so far. The DL-A complex is thus still the only example of autosomal linkage available in the dog.

In chapter 6 the results obtained are discussed and comparisons are made with the major histocompatibility complexes of other mammals. A system for DL-A nomenclature is proposed and explained. The possible functions of a major histocompatibility complex in transplantation immunology and biology in general are described. The many unknowns of the DL-A complex and its possible relation to other genetic systems, are listed. Discrepancies between results of histocompatibility testing in related and unrelated donor recipient pairs are given. A tentative explanation for differences in efficiency of matching for serologically defined antigens in dogs and primates is proposed.

Finally the need for further experiments is emphasized and suggestions for the most urgent ones are made.

SAMENVATTING

In het eerste hoofdstuk van dit proefschrift wordt een korte inleiding gegeven van het onderwerp histocompatibiliteit. Een historisch overzicht van belangrijke ontwikkelingen in twee verschillende gebieden van onderzoek wordt voor dit doel gebruikt, 1. experimentele transplantatie van maligne gezwellen en 2. de serologische determinatie van verschillen tussen individuen. Het tweede gedeelte van hoofdstuk 1 is gewijd aan de beschrijving van enkele van de huidige punten van onderzoek op het gebied van de histocompatibiliteit. Achtereenvolgens worden behandeld het aantonen van het bestaan van een hoofdhistocompatibiliteits complex, cellulaire methodes voor histocompatibiliteitsonderzoek en de biologische betekenis van histocompatibiliteit.

Hoofdstukken 2 tot en met 5 zijn reeds als afzonderlijke wetenschappelijke artikelen gepubliceerd. De in deze publicaties gebruikelijke korte vorm van rapportage, verdient nadere uitleg voor de oningewijde. Daarom dient hoofdstuk 1 tevens als een soort leidraad voor deze hoofdstukken.

In hoofdstuk 2 wordt de produktie en evaluatie van serologische histocompatibiliteits reagentia in honden beschreven. Het bestaan van een hoofd histocompatibiliteits systeem in de hond (DL-A) wordt aannemelijk gemaakt. Later (zie hoofdstuk 5 en 6) zal dit "systeem" gepromoveerd worden tot een "complex", aangezien het gecompliceerder blijkt te zijn, dan aanvankelijk was verondersteld.

In hoofdstuk 3 worden de eerste serologisch gedefinieerde antigenen van het DL-A complex beschreven. Het bewijs voor het bestaan van tenminste twee series van multiple allelen, die coderen voor DL-A antigenen, wordt geleverd.

Hoofdstuk 4 geeft meer informatie over de DL-groepen van hoofdstuk 3 en voegt verschillende groepen toe aan de reeds bekende. Recombinatie tussen de twee series wordt gevonden. Een positieve correlatie tussen sommige antigenen van de twee series in een populatie van onverwante honden wordt gevonden. Dit suggereert het bestaan van linkage disequilibrium tussen de twee series. Bij het ziektebeeld leukosis wordt DL-7 vaker aangetroffen dan verwacht, alhoewel deze resultaten nog bevestigd zullen moeten worden door verdere studies. Een over-

zicht van de overlevingsduur van orgaantransplantaten van donoren, die identiek zijn voor het hoofd histocompatibiliteits complex, wordt gegeven.
Hoofdstuk 5 is het verslag van de eerste internationale Workshop on Canine Immunogenetics die van 5 - 17 november 1972 gehouden werd aan de Medische Faculteit te Rotterdam. Eerder gedefinieerde DL groepen zijn bevestigd door de resultaten van de andere wetenschappelijke teams. Eén nieuwe DL groep is gevonden tijdens de Workshop. De nomenclatuur van Rotterdam-Leiden zal internationaal worden aangehouden op basis van de tijdens de Workshop bereikte overeenkomst tot een volgende Workshop. De aanwezigheid van nog een derde genetisch systeem in het DL-A gebied wordt aannemelijk gemaakt. Het verschilt van de reeds beschreven series van multiple allelen, die coderen voor serologisch herkenbare antigenen, en controleert de reactiviteit in gemengde lymfocytenkweken (mixed lymphocyte cultures).
Twee antigenen die op lymfocyten voorkomen, doch niet tot het DL-A complex behoren, worden gevonden. Zij zijn waarschijnlijk allelen van één en hetzelfde genetische systeem. Andere genetische merkgenen, als bloedgroepen, tractus digestivus groepen, polymorphe erythrocyten- en leucocyten enzymen en serum proteïne polymorphismen zijn ook tijdens de Workshop onderzocht. Geen aanwijzing voor nauwe koppeling tussen één van deze genetische systemen en DL-A is gevonden. Het DL-A complex blijft dus voorlopig het enige voorbeeld van autosomale koppeling in de hond.
In hoofdstuk 6 worden de verkregen resultaten besproken en vergeleken met de beschikbare gegevens over histocompatibiliteit in andere dieren. Een uitgewerkt voorstel voor DL-A nomenclatuur wordt gedaan en verdedigd. De mogelijke functies van een hoofd histocompatibiliteitscomplex in de transplantatie immunologie en de algemene biologie worden beschreven. Tevens worden de vele onzekerheden van het DL-A complex en zijn relatie met andere genetische informatie opgesomd. De verschillen in resultaten bereikt met weefseltypering in verwante en onverwante donor-ontvanger combinaties worden opgesomd en uitgelegd. Een mogelijke verklaring wordt gegeven voor het verschil in effectiviteit van donorselectie met serologische methoden in honden en primaten. De noodzaak voor verdere experimenten wordt uiteengezet.

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It is an established custom for the author of a thesis to end his script by elaborately thanking his or her wife or husband and family for the supportive

care they have given during the preparation of the thesis. This is, however, of no interest or concern to the readers of these lines, so I will here just say thank you to Francine, Corine, my parents and the rest of my family, including Mascha, for the patience, they have had with me.

CURRICULUM VITAE AUCTORIS

H.M. Vriesendorp was born November 1944 in Renesse, the Netherlands. In 1963 he passed the final examination of the β department of the Praedinius Gymnasium in Groningen. In the same year he became a medical student at the Leyden University. His interest for research in immunology was raised during the five months that he spent early in 1968 at the Radiobiological Institute TNO, Rijswijk, the Netherlands (Director Prof. Dr. D.W. van Bekkum). At the end of 1968 he finished the medical school of the University of Leyden and continued his medical education at the Medical Faculty Rotterdam. Early in 1969 the studies described in this thesis were started in the Laboratory of Experimental Surgery, Erasmus University Rotterdam (Head Dr. D.L. Westbroek), in close collaboration with the Department of Immunohaematology, Leyden University (Head Prof. Dr. J.J. van Rood). In March, 1971 he obtained his medical degree in Rotterdam and from that time onwards dedicated all his time to his experimental studies. In September 1972 he received the biennal prize of the "Klinisch Genootschap Rotterdam" for his experimental work. In January 1973 he joined the Primate Centre TNO, Rijswijk, The Netherlands (Director Dr. H. Balner).