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Cytogenetic Findings in Mouse Multiple Myeloma and Waldenström's Macroglobulinemia

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ABSTRACT: *Multiple myeloma (MM) and Waldenström's macroglobulinemia-like lymphoma (MW) appear spontaneously in C57BL/KaLwRij mice at a frequency of 0.5% and 0.2%, respectively. They can readily be propagated by intravenous transfer of mainly bone marrow or spleen cells into syngeneic recipients. Previous studies demonstrated that these mouse malignant monoclonal gammopathies (MMG) show clinical and biologic features that closely resemble those of the corresponding human diseases and thus could be used as experimental models. We report on cytogenetic analysis of two mouse MW and five MM in vivo cell lines of the 5TMM series propagated in syngeneic mice. These studies demonstrated clonal abnormalities in all cell lines, hyperdiploid karyotype in both MW and one MM lines, and hypotriploidy, hypertriploidy, or hypotetraploidy in the other lines. Structural abnormalities of chromosome 15 were observed in all MM lines. In the five MM lines, frequent rearrangements were also found for chromosome numbers 1, 2, 5, and 12. A single chromosomal abnormality, as found in induced mouse plasmacytomas and resembling Burkitt lymphoma, was not found in mouse MM and MW. It was concluded that spontaneously originating C57BL MM of the 5T series is a better model for human MM than pristane-induced BALB/c or NZB plasmacytoma.*

INTRODUCTION

Multiple myeloma (MM) and Waldenström's macroglobulinemia-like lymphoma (MW) appear spontaneously in mice of the C57BL/KaLwRij strain at a frequency of about 0.5% and 0.2%, respectively [1, 2]. Both these malignant monoclonal gammopathies (MMG) of the 5T series can readily be propagated by intravenous transfer of mainly bone marrow or spleen cells into recipients of the same strain. Until now, ten different transplantable mouse 5T lines have been developed. They differ substantially from mouse plasmacytomas originating after pristane injection in BALB/c mice [3]: in the bone marrow involvement, including the bone disease, in the way of growth, homing, and some other biologic features [1, 2]. So far, they were found to resemble their corresponding human diseases and therefore will possibly be useful as an experimental model.

In human MM a variety of chromosome abnormalities were found. Frequent features were trisomy 3, 5, 7, 9, 11, 15, and 21, monosomy 8 and 13, as well as structural abnormalities of chromosomes 1 and 14, and some not fully identified genetic markers [4–8]. In human MW hyperdiploidy with restricted numerical and structural abnormalities plus one or two markers was reported [9–11]. Induced mouse plasmacytomas showed most resemblance to human Burkitt lymphoma [3]. In both latter conditions a specific reciprocal translocation involving the *MYC* oncogene and the immunoglobulin heavy or light chain locus was ob-served.

In the present study, we investigated whether chromosome abnormalities occurred in the mouse 5T lines and, if so, whether the mouse MM and MW lines resembled human MM and MW or mouse plasmacytoma. Further, the karyotype of two different transfer generations, i.e., 6 and 26, of the mouse 5T2MM was investigated for similarity of markers.

MATERIALS AND METHODS

Mouse Model of MMG (5T Model)

Spontaneously originating mouse multiple myeloma and Waldenström's macroglobulinemia-like lymphoma have been maintained and propagated by intravenous transfer of bone marrow or spleen cells in syngeneic recipients of the C57BL/KaLwRij strain [1, 2] (Table 1).

For cytogenetic analysis, cells from 10 different 5T MMG lines of different in vivo generation numbers were injected into mice of the sex opposite to that of the mouse

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Table 1 Characteristic features of the C57BL/KaLwRij MMG lines karyotyped

Line MM/MW	Isotype M-protein	Transfer generation	Sex mouse		Bone marrow involvement	Bone lesions	Growth Pattern
			Primary	Recipient			
5T2 MM	IgG2aK	6	M	F,F	+	++	Moderate
		26		F	+	++	
5T7 MM	IgG2bK	7	M	F	+	−	Smoldering
5T13 MM	IgG2bK	7	M	F,F	+	+	Moderate
5T14 MM	IgG1K	10	M	F,F	+	+[a]	Aggressive
		11		F,F	+	+[a]	
5T21 MM	IgDK	10	F	F,F	+	+	Atypical
5T30 MM	IgG2aK	6	F	M	+	+	Aggressive
5T33 MM	IgG2bK	5	F	F,F	+	+	Moderate
		6		F	+	+	
		11		M	+	+	
5T10 MW	IgMK	7	M	F,F	+	−	Moderate
5T16 MW	IgMK	12	M	F,F	+	−	Aggressive
5T18 MW	IgMK	10	M	F,F	+	−	Aggressive

[a]Mainly osteolytic lesions; in some sublines, also osteosclerotic.

in which the primary tumor originated. Because of restrictions in the availability of recipient mice of the appropriate sex, the 5T21 and 5T33 cell lines were also injected in a few instances into mice of the same sex as was that of the primary MMG carrier.

Successful take was verified by testing the immunoglobulin isotype of the homogenous immunoglobulin band in the serum by high-resolution electrophoresis and immunofixation using appropriate specific antisera [12].

Cytogenetic Technique

Five weeks to 14 months after cell transfer (dependent on the aggressivity of the 5T MMG line), the recipient mouse was sacrificed. Bone marrow, spleen and mesenteric lymph nodes were removed, minced, and put into culture for 20 to 24 hours (RPMI medium, 10% fetal calf serum, 37°C). A pulse of 10^{-5} M BrdU was given 6 hours before harvesting. Colcemid (10 μg/mL) was added one minute before harvesting. Harvesting of metaphases was done

Table 2 Summary of cytogenetic data obtained from in vivo passaged mouse MMG cell lines (5T model)

MMG lines			Sex of recipient mice	Tissue karyotyped	Number of metaphases and karyotypes obtained			
Name	Sex	Transfer generation			Recipient origin		Tumor origin[a]	
5T2	M	6	F,F	BM[b] (2x), spleen (2x)	12	40, XX	20	62–65, hypertriploid
		26	F	BM, spleen, node[b]	15	40,XX	11	60, pseudotriploid
5T7	M	7	F	BM, spleen	40	40,XX	5	64–66, hypertriploid
5T13	M	7	F,F	BM (2x)	21	40,XX	0	
5T14	M	10	F	BM	22	40,XX	0	
			F,F	BM (2x), spleen (2x)	7	40,XX	23	58–59, hypotriploid
5T21	F	10	F,F	BM (2x)	48	40,XX	0	
5T30	F	6	M	BM	22	40,XY	2	42–43, specific markers
5T33	F	5	F	BM, spleen	4	40,XX	0	
		6	F	BM	5	40,XX	5	76–79, hypotetraploid
		11	M,M	BM, spleen	25	40,XY	0	
5T10	M	7	F,F	BM (2x), spleen (2x), node (2x)	44	40,XX	9	42–43, specific markers
5T16	M	12	F,F	BM (2x)	23	40,XX	0	
5T18	M	10	F,F	BM (2x), spleen (2x)	20	40,XX	11	42–43, specific markers

[a]For description of modal karyotype of tumor line, see Table 3.

[b]BM, bone marrow; node, mesenteric lymph node.

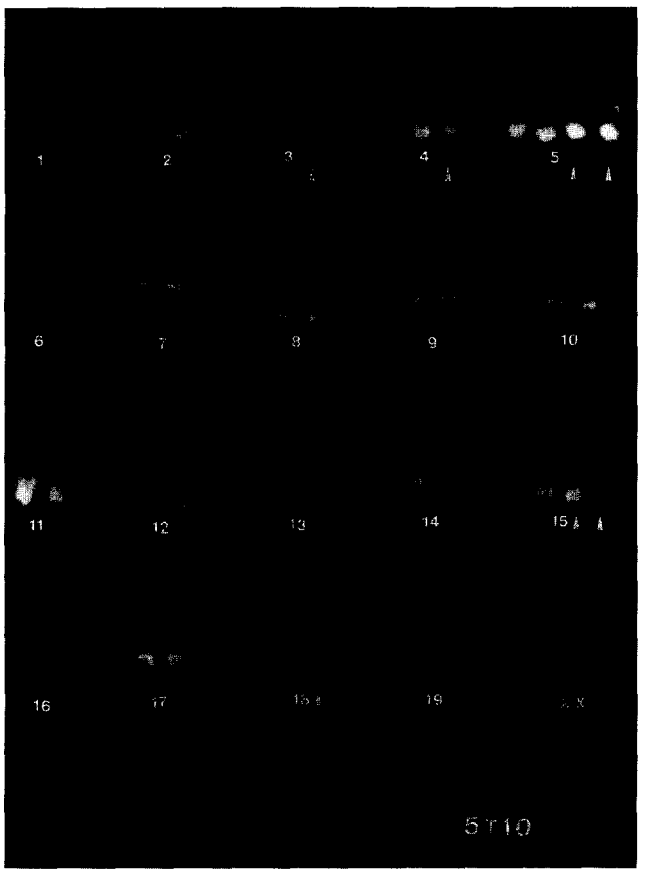
Table 3 Modal karyotype of the mouse MMG cell line passaged in vivo

Line MM/MW	Sex tumor	No. of cells	Modal karyotype
5T2	M	20	62–65,<3n>[a]XX,−Y?,mar1,2q$^+$,inv(2),−4,(−4),+4q$^+$(x3),5q$^-$,−5,7q$^+$,(−8),−9,−11, 12p$^+$,14q$^-$,rcp t(15;15)(q$^+$;q$^-$),robt(5;15),+18q$^-$,19q$^+$,+4–6 markers
5T7	M	5	64–66,<3n>[a]XX,−Y?,der(2),+6,−7,−17,−19,+mar1[robt(1;7)],+mar2[rob t(12;7)], +mar3[tan t(18;9 or 10)],+2mar4[der(inv15?)]+mar5[2p+],+2 small ring (Y?)
5T14	M	23	58–59,<3n>[a],XX,−Y,+1,ins(2)(50%) or 2q$^-$(50%),−4,−5,5q$^-$,−8,−12,−14,15q$^-$, +15q$^-$,+16,+17,+ large mar [?t(12;6)],+very small marker (NB: some intercellular variation in number of copies of normal chromosomes)
5T30	F	2	42–43,X,−X?,t(1;9),der(6),t(12;14),t(12;19),+13,+der(15),+17,+small marker
5T33	F	5	76–79,<4n>[a],XX,−X,−X,t(1;15),t(1;15),inv(5),inv(5),−8,−10,−15,−17,+2 mar
5T10	M	9	41–43,X,−Y?,+X,2q$^-$,3q$^+$,4q$^+$,+5,(+5),der(15),+der(15),18q$^-$,+19(50%),+mar(50%) (NB: 3q$^+$ and 4q$^+$ = der of t(3;4;15) with possibly partial trisomy 15)
5T18	M	10	42–43,X,marY?,5q$^+$,8q$^+$,18q$^-$,19q$^+$,+der t(16?;X),+1 or 2 small markers

[a]<3n>,<4n>, in angle brackets is the ploidy number, e.g., 3n, triploidy; 4n, tetraploidy. Numerical changes are given relative to a triploid or tetraploid karyotype, respectively.

according to standard procedure. Chromosomes were identified using R-, Q-, and sometimes also G-bands and classified according to nomenclature for mouse chromosomes [13].

Figure 1 R-banded karyotype of one 5T10 MW metaphase containing 43 chromosomes. Arrowheads indicate markers.



RESULTS

Characteristic features of the 10 C57BL/KaLwRij MMG lines that were investigated are shown in Table 1. With the followed methodology more aggressive MM lines, especially the fast-growing 5T2, 5T14, and 5T33MM lines, could be karyotyped more easily than the slow-growing ones (5T7, 5T21). A summary of cytogenetic data of the mouse MM and MW cell lines is given in Tables 2 and 3. Cytogenetic analysis of in vivo transferred lines proved to be possible but presented some limitations compared to a study of in vitro established lines. Major complications were the admixture of normal recipient cells which at the time constituted the majority of the metaphases (Table 2), the limited number of metaphases, and the time (a few months) required to repeat an experiment. To obviate these difficulties the recipient mouse used was usually of the opposite sex as the mouse in which the primary tumor originated and in some cases different animals were inoculated at the same time with the same pool of tumor cells.

It is noticeable that bone marrow specimens showed better chromosome morphology but a lower percentage of tumor metaphases than spleen, which showed poor chromosome morphology but a higher percentage of tumor metaphases. Mesenteric lymph node samples (5T2, 5T10) showed almost exclusively tumor cells. All metaphases from recipient origin showed a normal diploid karyotype (40, XX and 40, XY) with infrequent random loss of 1 or 2 chromosomes; chromosome breaks and translocations were very exceptional. In three of the lines (5T13, 5T21, and 5T16) only the recipient's own cells were found. The mouse MMG cell lines were characterized by aneuploidy and clonal abnormalities (Table 3), consistent in different animals injected with the same tumor lines, even after a number of in vivo transfers (5T2). Most of the tumor lines were of male origin, but they showed loss of Y chromosomes and thus the discrimination between recipient and tumor metaphases based on sex chromosomes was less reliable than expected.

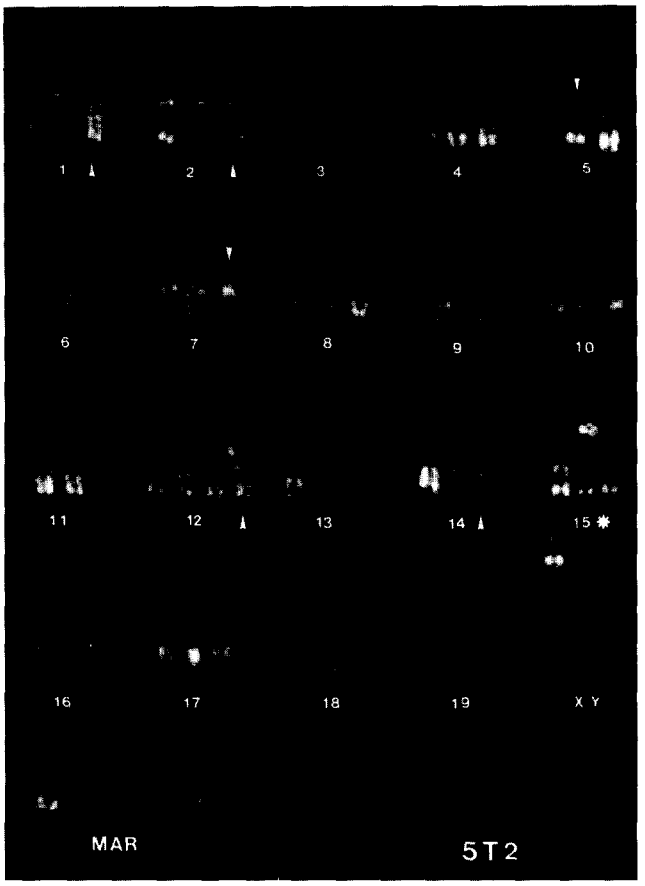


Figure 2 R-banded karyotype of one 5T2 MM metaphase containing 63 chromosomes. The majority of markers are indicated by arrowhead. There is no normal chromosome 4 but 3 copies of $4q^+$, one with a break near the centromere; there is no normal chromosome 15; the $18q^-$ and the $19q^+$ were not seen in this particular cell.

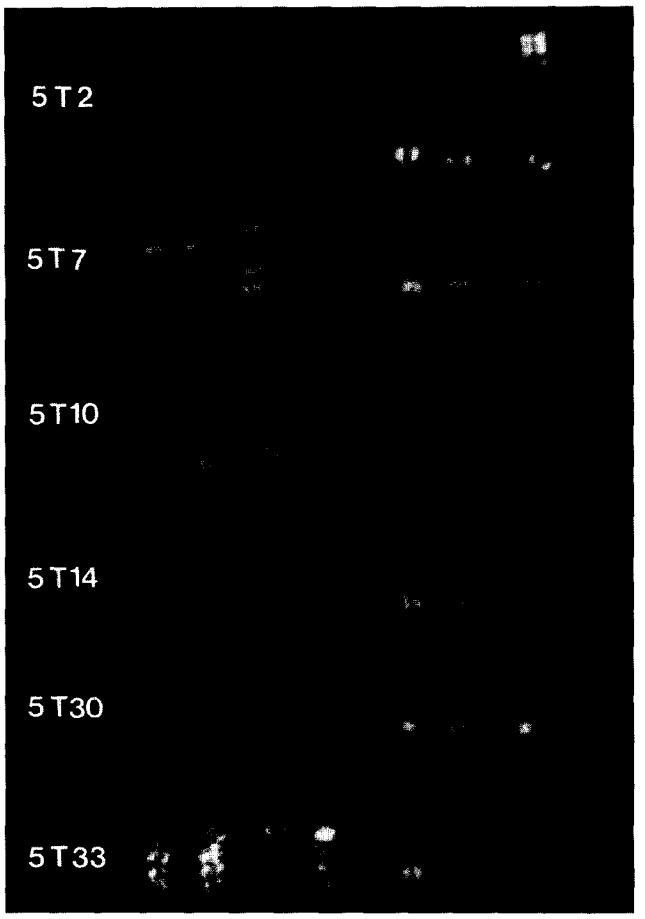


Figure 3 Partial karyotype (R-bands) of the 5T cell lines showing rearrangements of chromosome 15. 5T2, rcp t(15;15)(?B;?D3) and Rb(5;15); 5T7, two copies of mar 4 interpreted as inv (15), one copy of mar 5 interpreted as der (2;5) and three copies of chromosome 15; 5T10, chromosome pairs 3 and 4 showing t(3;4;15) (G;E;?A) and two normal chromosome 15; 5T14, two normal 15 and two del(15)(D?3); 5T30, two normal 15 and a der (15); 5T33, two normal 1, two der(15;1)(E;C), and three normal 15.

The two MW lines, 5T10 (Fig. 1) and 5T18, resembled each other somewhat cytogenetically. Hyperdiploidy with 42–43 chromosomes, $18q^-$, numerical or structural abnormalities of chromosome 5 and 19, plus one or two small markers were shared features of the two MW lines. In human MW, hyperdiploidy with restricted numerical and structural abnormalities plus one or two markers have been reported [9–11], observations similar to the findings in the 5T10 and 5T18 MW lines.

In the 5T MM lines a variety of chromosomal abnormalities was found (Table 3). A hyperdiploid number of chromosomes was detected in 5T30. Here, only two cells could be analyzed. Hypotriploidy with 58–59 chromosomes was found for the 5T14 MM cell line. In two cases, 5T2 (Fig. 2) and 5T7, hypertriploidy with 62–66 chromosomes was detected. Hypotetraploidy with 76–79 chromosomes was found for the 5T33 cell line. In general, male mouse MM tumor cells tended to lose the Y-chromosome. Structural changes of chromosomes were variable. Chromosome 15, where the *MYC* oncogene was mapped in the 15D2/D3 region [14], was rearranged in all five lines (Fig. 3). These rearrangements resulted in overrepresentation of (part of) chromosome 15 without specificity, apparently, and as far as can be determined at the banding level. Further, frequent alterations were also found for chromosome numbers 1, 2, 5, and 12, in addition to the numerous numerical changes.

Since no common cytogenetic characteristics were detected among the MM lines, the known biologic variations of the MM lines could not be related to chromosome abnormalities. Thus, the 5T7 line that clinically behaves like a smoldering MM and 5T14 line with both lytic and osteosclerotic bone lesions could not be distinguished cytogenetically from the other MM lines. The 5T2 MM cell line was karyotyped at transfer generations 6 and 26. The latter showed cells of less quality than the former, a slight decrease in modal chromosome number but persistence of the same characteristic markers.

DISCUSSION

The chromosome abnormalities found in the mouse MM lines are similar to those in human MM. Frequent features

in human MM were trisomy 3, 5, 7, 9, 11, 15, and 21, monosomy 8 and 13, several structural abnormal chromosomes, and some not fully identified markers [4–8]. Characteristic translocations as are known for Burkitt lymphoma [15] in humans and in plasmacytoma in mouse [3] were not observed. Mouse plasmacytomas, induced by pristane injection of BALB/c or NZB mice, consistently contain one of two alternative translocations, the typical rcpt (12;15) or the variant rcp t(6;15) (3, 14). The breakpoint on chromosome 15 in plasmacytomas was found to map in the D2/D3 region where the *MYC* oncogene is located [14]. Using Southern blot technique DNA rearrangement of the *MYC* oncogene was consistently detected in mouse plasmacytomas [14]. In the 5T series, chromosome 15 was involved in chromosomal aberrations in all MM lines. However, here no DNA rearrangement of the *MYC* oncogene was found by Southern blot analysis of spleen cells from all 5T MM-bearing mice investigated [17, 18]. In 5T2 bone marrow and in 5T2 and 5T14 ascitic cells rearrangement of the *MYC* oncogene was detected [17, 18]. In human MM, rearrangement of the *MYC* oncogene was found only in exceptional cases, such as in a very progressive IgA-MM involving pleural tissue and in a case of plasma cell leukemia [19–21]. These data of mouse and human MM can be interpreted as indicating that *MYC* rearrangement in MM can take place, possibly as a late event in the progression of this malignancy. Thus, *MYC* rearrangement is not a consistent feature of mouse and human MM, in contrast to mouse plasmacytoma. On the basis of these great differences of cytogenetic and molecular genetic data between C57BL MM and BALB/c plasmacytoma, we conclude that spontaneously arising C57BL MM and pristane-induced plasmacytoma are different tumors. Comparing both biologic [16] and cytogenetic data of human MM with C57BL MM and BALB/c plasmacytoma we further conclude that C57BL MM of the 5T series is an appropriate model for human MM, in contrast to BALB/c plasmacytoma.

In this study we could show major karyotypic changes in mouse MW and MM lines of the 5T series. These cytogenetic abnormalities were clonal and relatively stable characteristics of each individual lines. This indicates that, in the future, molecular genetics and molecular cytogenetic technical approaches can be applied to investigate these lines for specific gene rearrangements and alterations of gene expression. In conclusion, our data confirm other observations on the close resemblance of the cytogenetic characteristics of human MM and MW on the one hand, and mouse MM and MW 5T lines on the other. This suggests that these MMG of the 5T series may offer a good experimental model for studies on several aspects of MM and MW.

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REFERENCES

1. Radl J, Croese JW, Zurcher C, Van den Enden-Vieveen MHM, De Leeuw AM (1988): Animal model of human disease: Multiple myeloma. Am J Pathol 132:593–597.
2. Radl J, Van den Enden-Vieveen MHM, Zurcher C, Roholl PJM, Blauw E (1989): Morbus Waldenström-like B-cell lymphoma in the aging C57BL/KaLwRij mouse. In: Radl J, Hijmans W, Van Camp B (eds) Monoclonal Gammapathies — Clinical Significance and Basic Mechanisms; Topics in Aging Research in Europe. Vol. 12 Eurage, Rijswijk, pp. 233–238.
3. Potter M (1986): Plasmacytomas in mice. Semin Oncol 13: 275–281.
4. Gould J, Alexanian R, Goodacre A, Pathak S, Hecht B, Barlogie B (1988): Plasma cell karyotype in multiple myeloma. Blood 71:453–456.
5. Dewald GW, Kyle RA, Hicks GA, Greipp PR (1985): The clinical significance of cytogenetic studies in 100 patients with multiple myeloma, plasma cell leukemia or amyloidosis. Blood 66:380–390.
6. Lewis JP, MacKenzie MR (1984): Non-random chromosomal aberrations associated with multiple myeloma. Hematol Oncol 2:307–317.
7. Chen K-C, Bevan PC, Matthews JG (1986): Analysis of G-banded karyotypes in myeloma cells. J Clin Pathol 39:260–269.
8. Ranni NS, Slavutski I, Wechsler A, Brieux S (1987): Chromosome findings in multiple myeloma. Cancer Genet Cytogenet 25:309–316.
9. Han T, Sadamori N, Takeuchi J, Ozer H, Henderson ES, Bhargava A, Fitzpatrick J, Sandberg AA (1983): Clonal chromosome abnormalities in patients with Waldenström's and CLL-associated macroglobulinemia: significance of trisomy 12. Blood 62:525–531.
10. San Roman C, Ferro T, Guzman M, Odriozola J (1985): Clonal abnormalities in patients with Waldenström's macroglobulinemia with special reference to a Burkitt-type t (8;14). Cancer Genet Cytogenet 18:155–158.
11. Palka G, Spadano A, Geraci L, Fioritoni G, Dragani A, Calabrese G, Guanciali Franchi P, Stuppia L (1987): Chromosome changes in 19 patients with Waldenström's macroglobulinemia. Cancer Genet Cytogenet 29:261–169.
12. Radl J (1982): Effects of aging on immunoglobulins. In: Ritzmann SE (ed). Protein Abnormalities, Vol. 2. Pathology of Immunoglobulins - Diagnostic and Clinical Aspects. Alan R. Liss Inc, New York, pp. 55–69.
13. Nesbitt MN, Francke U (1973): A system of nomenclature for band patterns of mouse chromosomes. Chromosoma 41:145–158.
14. Wiener F, Ohno S, Babonits M, Sümegi J, Wirschubsky Z, Klein G, Mushinsky JF, Potter M (1984): Hemizygous interstitial deletion of chromosome 15 (band D) in three translocation-negative murine plasmacytomas. Proc Natl Acad Sci USA 81:1159–1163.
15. Klein G (1983): Specific chromosomal translocations and the genesis of B-cell derived tumors in mice and men. Cell 32:311–315.
16. Radl J, Croese JW, Zurcher C, Brondijk RJ, Van den Enden-Vieveen MHM (1985): Spontaneous multiple myeloma with bone lesions in the aging C57BL/KaLwRij mouse as a natural model of human disease. In: Radl J, Hijmans W, Van Camp B (eds). Monoclonal Gammapathies — Clinical Significance and basic mechanisms; Topics in Aging Research in Europe. Vol. 5 Eurage, Rijswijk, pp. 191–194.
17. Radl J, Punt YA, Van den Enden-Vieveen MHM, Bentvelzen PAJ, Bakkus MHC, Van den Akker ThW, Benner R (1990): The 5T mouse multiple myeloma model: Absence of c-*myc* oncogene rearrangements in early transplant generations. Br J Cancer 61:276–278.

18. Van den Enden-Vieveen MHM, Punt YA, Bakkus M, Benner R, Radl J (1989): Rearrangement of the c-myc oncogene in mouse multiple myeloma, Morbus Waldenström and plasmacytoma. In: Radl J, Van Camp B (eds). Monoclonal Gammapathies II., Topics in Aging Research in Europe, Vol. 12 Eurage, Rijswijk, pp. 245–248.

19. Gazdar AF, Oie HK, Kirsch IR, Hollis GF (1986): Establishment and characterization of a human plasma cell myeloma culture having a rearranged cellular *myc* proto-oncogene. Blood 67:1542–1549.

20. Selvanayagam P, Blick M, Naini F, Van Tuinen P, Ledbetter DH, Alexanian R, Saunders GF, Barlogie B (1988): Alteration and abnormal expression of the c-*myc* oncogene in human multiple myeloma. Blood 71:30–35.

21. Yamada K, Shionoya S, Amano M, Tinamura Y (1983): A Burkitt type 8;14 translocation in a case of plasma cell leukemia. Cancer Genet Cytogenet 9:67–70.