ELSEVIER

Journal of Immunological Methods 194 (1996) 13–25

JOURNAL OF IMMUNOLOGICAL METHODS

Immunological standardization

Implications for the assay and biological activity of interleukin-4 Results of a WHO international collaborative study

Anthony R. Mire-Sluis [a,*], Rose Gaines Das [b], Robin Thorpe [a], Participants [1] of the Study

[a] *Division of Immunobiology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG, UK*

[b] *Informatics Laboratory, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts, EN6 3QG, UK*

Received 25 January 1996; accepted 19 March 1996

Abstract

Five ampouled preparations of interleukin-4 (IL-4) have been evaluated by 36 laboratories in 14 countries for their suitability to serve as an international standard for this material in a joint international collaborative study for interleukin-3 (IL-3) and IL-4. The preparations were assayed in a wide range of in vitro bioassays and immunoassays. It is clear from the study that different recombinant preparations of IL-4 can have very different biological specific activities, including those from the same source (i.e., *E. coli*). In addition, immunoassay estimates of IL-4 levels did not correlate with those of bioassays, illustrating the fact that immunoassays do not necessarily measure biologically active cytokine. It is of interest that the estimates provided by the different bioassays were less variable than those produced by the immunoassays, suggesting that bioassays can be as accurate, if not more so, than immunoassays. The large reduction in the variability of estimates with the inclusion of a single reference preparation clearly illustrates the need for a single standard to assay IL-4. On the basis of the results reported here, with the agreement of the participants of the study and with the authorisation of the Expert Committee on Biological Standardization (ECBS) of the World Health Organization (WHO) the preparation of IL-4 (88/656) was established as the international standard for interleukin-4 with an assigned unitage of 1000 IU/ampoule.

Keywords: IL-4; Standardization

1. Introduction

Interleukin-4 (IL-4) is a T lymphocyte derived polypeptide possessing a broad spectrum of biological activities (Howard et al., 1982; Yokota et al., 1988). In addition to inducing proliferation of human B cells, it can stimulate the proliferation of a wide range of cells such as T lymphocytes, mast cells and haemopoietic progenitor cells (Ohara, 1988). The gene for IL-4 has been cloned and shown to code for a 129 amino polypeptide which is glycosylated and has a molecular mass of 15–22 kDa (Yokota et al., 1986; Ohara et al., 1987). IL-4 also has the ability to activate anti-tumour macrophages and inhibit the

Abbreviations: IL-3, interleukin-3; IL-4, interleukin-4; Rh, rDNA derived human; HSA, human serum albumin.

* Corresponding author. Tel.: +44-(707)654753; Fax: +44-(707)646730.

[1] Listed in Table 2.

0022-1759/96/$15.00 Published by Elsevier Science B.V.
PII S0022-1759(96)00069-5

growth of several different types of tumour cells such as lung tumours, renal cell carcinoma and B lymphomas (Golumbek et al., 1991; Defrance et al., 1992; Toi et al., 1992; Topp et al., 1993). This has led to clinical investigations of the therapeutic anti-cancer potential of IL-4 (Gilleece et al., 1992; Margolin et al., 1994).

The majority of bioassays for IL-4 utilise cell lines and thus avoid the donor variation of natural cell preparations (Avanzi et al., 1988; Siegel and Mostowski, 1990). There are several methods for detecting the activity of IL-4 due to its plietotropic biological properties. IL-4 can induce proliferation, inhibit proliferation, induce expression of proteins on the cell surface and promote secretion of proteins extracellularly (Avanzi et al., 1988; Topp et al., 1993; Siegel and Mostowski, 1990).

This collaborative study included several different cell line based assays, assays using natural material and immunoassays.

2. Aims of the study

The aims of the study were to:

1. assess the suitability of the ampouled preparations to serve as international standards (IS) for the bioassay and immunoassay of IL-4;
2. to assay the IL-4 content of the ampouled preparations;
3. to compare the different assay systems for IL-4;
4. to compare the ampouled preparations with local standards.

3. Materials and methods

3.1. Materials for the study

Generous gifts of rDNA derived human rhIL-4 were kindly donated by Immunex (USA), Schering Plough (USA), Innogenetics (Belgium), Sandoz (Switzerland) and Glaxo (UK) and rhIL-3 donated by Immunex (USA). The candidate international standards were ampouled according to the procedures used for international biological standards (Annex 4, 40th ECBS Report, 1990) and coded as detailed in Table 1.

Each preparation of rhIL-4 or rhIL-3 was dissolved in 2 or 4 litres of pyrogen-free saline (0.9% NaCl) pH 7.2 (Travenol Laboratories, Thetford, Norfolk, UK) containing 0.2% clinical grade human serum albumin and 0.1% trehalose (Fluka Chemicals, Glossop, Derbyshire, UK). This solution was distributed in 0.5 ml or 1 ml aliquots into 4000 ampoules. The ampouled solution was lyophilised and, after secondary desiccation, the ampoules were sealed under dry nitrogen by heat fusion of the glass and stored at −20°C in the dark.

88/656 has been distributed for use as an interim reference reagent with an assigned unitage of 1000 U/ampoule.

3.2. Participants in the study

48 laboratories in 16 countries contributed data as part of a joint IL-3 and IL-4 collaborative study (Table 2). Of these participants, 16 provided assays for both the IL-3 and IL-4 preparations, 12 provided assays for IL-3 preparations only, and 20 provided assays for IL-4 preparations only. In addition, one laboratory (NIBSC) provided additional data on stability. Throughout this report of the study, each laboratory has been identified by a number from 01 to 48, not related to the order of listing in Table 2. Results reported here include only those from assay systems which were either specific for IL-4 or which gave responses to both IL-3 and IL-4.

3.3. Design of the study

Participants were asked to contribute assays for IL-4. These were to include both bioassays and immunoassays, especially if the latter were unique to the laboratory of the participant with respect to the antibodies used.

Each participant was asked to carry out at least two independent assays each including, as far as possible, all of the preparations to be tested. For this study assays were considered independent if the dilutions of the various materials were made from a freshly prepared ampoule or fresh dilutions from an appropriately stored stock solution. Participants were requested to include preferably no less than five dilutions of each ampoule in the linear portion of the dose-response curve.

Table 1
Material included in the study

Study code	Ampoule code	Residual moisture content	Coefficient of variation	Nominal IL-3 or IL-4 content of ampoule
F	88/780	0.33%	0.21%	1 μg yeast IL-3 Pro_8 $Asp_{15/70}$
G/M	88/656	0.20%	0.15%	100 ng CHO IL-4
H	88/682	0.26%	0.16%	100 ng *E. coli* IL-4
J	89/508	0.33%	0.11%	1 μg yeast IL-4 Glu_1 Ala_2 Glu_3 Ala_4 $Asp_{38/105}$
K	89/668	0.15%	0.13%	1 μg *E. coli* IL-4
L	92/576	0.24%	0.17%	100 ng *E. coli* IL-4

The coefficient variation (CV) is defined as: CV = standard deviation ÷ mean (ECBS Technical Report Series 800, 1990, pp. 181–208).

Participants were asked to contribute all raw data in standard format together with detailed descriptions of assay methods, including their own calculated estimates of potency.

Included among the coded ampoules were unlabelled ampoules containing formulation without the active component in order to highlight non-specific effects in the assays.

3.4. Assays contributed to the study

Details of the assay methods used in the study are shown in Table 3 as extracted from the submissions of the participants. References to the methods used are included in the Reference list.

3.5. Statistical analysis

For each assay, the raw responses were plotted against the dilution of the preparation and examined both graphically and using analysis of variance. Groups of raw responses obtained using the same dilution of the same preparation in the same assay were examined for outliers both in terms of the within group variation and in terms of the variation of that group relative to the variation of other groups in the same assay (using the in-house program SCAN (Gaines Das and Rice, 1985)).

In most cases the IL-4 preparations showed a significant regression on dose with a relationship which could be reasonably described using a four parameter logistic function. Where the response showed no regression on dose, as was generally the case for the excipient or for the IL-3 preparations in an assay system which was specific for IL-4 preparations, all such responses were omitted from further analysis. In those assay systems where both IL-3 and IL-4 preparations gave responses which showed a significant relationship with dose, but where the relationship with dose differed markedly between the two types of preparation, the response data for the IL-3 preparation were also deleted from further analysis.

Where assays were carried out on microtitre plates, and only a single plate was used or each plate included a common standard, individual responses were used for analysis and responses from the single plate were analysed as an assay. If such assays extended over several plates, estimates from different plates for the same preparation were combined as geometric means to give a single estimate for each preparation from each assay. Where assays extended over several plates, each of which did not include a common standard, no allowance could be made for a possible difference between plates and the individual responses from the several plates have been analysed together as a single assay. Some laboratories used a degree of replication of plates and independent dilution series within and between plates; for these assays analysis has been carried out using the mean response within a plate or dilution series.

For other assays, for which detailed information about the assay design was generally limited, all individual responses have been combined and analysed as an assay.

Values for the upper and lower asymptotes of the four parameter logistic function were used to transform the raw responses to logit responses which were linearly related to log dose. These transformed responses were then analysed by weighted linear

Table 2
Participants in the study

Ms. Sandra Thomas, Virology Section, Therapeutic Goods Administration Laboratories, P.O. Box 100, Woden, ACT 2606, Australia
Dr. Darryl Maher, Ludwig Institute, Melbourne Tumour Biology Branch, Post Office, Royal Melbourne Hospital, Melbourne, VIC 3050, Australia
Dr. Peter Valent, Department of Internal Medicine I, Division of Hematology and Hemostaseology, University of Vienna, Währinger Gürtel 18–20, A-1090 Vienna, Austria
Dr. Elisabeth Payer, VIRCC, Division of Dermatology, University of Vienna Medical School, Brunner Strasse 59, A-1235 Vienna, Austria
Dr. De Groote, Medigenix Diagnostics, Zoning Industrial, 6220 Fleurus, Belgium
Dr. Kent Hayglass, Department of Immunology, University of Manitoba, Basic Medical Sciences Building, 730 William Avenue, Winnipeg, Manitoba R3E OW3, Canada
Dr. H. Ziltener, The Biomedical Research Center, 2222 Health Sciences Mall, UBC Vancouver, British Columbia V6T 1Z3, Canada
Dr. Lars K. Poulsen, National University Hospital, Laboratory of Medical Allergology, Medical Department TTA 7542, Tagnensvej 20, DK-2200 Copenhagen, Denmark
Dr. Pierre Miossec and Miss P. Chomarat, Department of Immunology and Rheumatology, Hôpital Edouard Herriot, 69437 Lyon Cedex, France
Dr. Jacques Grassi, SPI/DRIPP, Bâtiment 136, Commissariat à l'Energie Atomique, CEN Seclay, 91191 Gif-sur-Yvette, Cedex, France
Dr. G. Pawelec, Medizinische Klinik, W-72076 Tübingen, Germany
Dr. U. Fritzsche, Behringwerke AG, Preclinical Research, P.O. Box 1140, Emil-van-Behring-Strasse 76, W-3550 Marburg/Lahn, Germany
Dr. M. Falk, Department of Cell Biology, IBR Forschungs GmbH, Südkampen 31, D-3030 Walsrode, Germany
Dr. M. Topp, Department of Haematology and Oncology, Benjamin Franklin Klinik, Freie Universität Berlin, Hindenburgdamm 30, 12200 Berlin, Germany
Dr. G. Reisbach, GSF Forschungszentrum, Hämatologikum, Marchionini Strasse 25, D-81377, Munich, Germany
Dr. John Sidiropoulos, Clinical Immunology Department, 2nd Medical Department/University, Hippokration Hospital, Thessaloniki, Greece
Dr. S.Z. Ben-Sasson, The Lautenberg Center for General and Tumour Immunology, The Hebrew University – Hadassah Medical School, P.O. Box 1172, Jerusalem 91010, Israel
Dr. Zeev Even-Chen, Sigma Israel Chemicals Ltd. (formerly Makor Chemicals Ltd.), P.O. Box 6570, Jerusalem 91064, Israel
Dr. Luigi Pegoraro, Dipartimento di Scienze Biomediche e Oncologia Umana, Sezione Clinica, Via Genova 3, 10126 Turin, Italy
Professor Francesco Barboni, Director of Clinical Pathology, Malpighi Hospital, Via P. Palagi 9, 40138 Bologna, Italy
Dr. Kenkichi Sugimoto, Department of Physiology and Biochemistry, Faculty of Science, Niigata University, Niigata 950-21, Japan
Dr. K. Tsuji, Department of Pediatrics, Shinsu University School of Medicine, 3-1-1 Asahi, Matsumoto, Japan
Dr. Tomoaki Matsumoto, Department of Child Development, Kumamoto University, Medical School, 1-1-1 Honjo, Kumamoto 860, Japan
Drs. Kenrichi Arai and Takashi Yokota, Department of Molecular and Developmental Biology, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo, Japan
Dr. J. Olobo, Institute of Primate Research, P.O. Box 24481, Karen, Nairobi, Kenya
Dr. G. Wagemaker, Erasmus Universiteit Rotterdam, Hematology, Room H EE 1314, P.O. Box 1738, 3000 DR Rotterdam, Netherlands
Dr. T. Van Der Pouw-Kraan, Central Laboratory of the Netherlands, Red Cross Blood Transfusion Service, Plesmanlaan 125, 1066 CX Amsterdam, Netherlands
Dr. Kirrikant V. Sheth, Head Immunopathology Section, King Faisal Special Hospital and Research Centre, Department of Pathology and Laboratory Medicine, P.O. Box 3354, Riyadh 11211, Saudi Arabia
Drs. C. Gutierrez and D. Rivas, Head – Department of Immunology, Hospital Central de Asturias, Celestino Villamil, s/n 33006 Oviedo, Spain
Drs. Nils Lycke and Karin Schön, Department of Medical Microbiology and Immunology, University of Göteborg, Göteborg, Sweden
Dr. John P.A. Bews, CIBA-GEIGY Pharma, R1056.408, CH-4002 Basel, Switzerland
Dr. K. Tullberg, Sandoz AG, Bau 360/307, Postfach, CH-4002 Basel, Switzerland
Dr. R. Klotzbücher, Roche Diagnostic Systems, Department DDIR-T, Building 205/ab, P.O. Box, CH-4002 Basel, Switzerland
Mrs. L. Page and Mr. C. Bird, Division of Immunobiology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts. EN6 3QG, UK
Dr. Colin P. McGuckin, Haematology Division, St. George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE, UK
Professor John Gordon and Miss Anita Katira, Department of Immunology, The Medical School, Vincent Drive, Birmingham B15 2TT, UK
Dr. Peter Openshaw and Andrew Georgiou, Department of Medicine, St. Mary's Hospital Medical School, Norfolk Place, London W2 1PC, UK
Dr. Nydia G. Testa, Paterson Institute for Cancer Research, Christie Hospital, Manchester M20 9BX, UK

Table 2 (continued)

Dr. Robin Callard, Cellular Immunology Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1AH, UK
Ms. K. Paik and Dr. John McKearn, Searle, 700 Chesterfield Village Parkway, St. Louis, MO 63193, USA
Dr. Marie Rock and Ms. Kathleen Harper, Searle, 4901 Searle Parkway Room K-224, Skokie, IL 60077, USA
Dr. Steven J. Swanson, Associate Principal Scientist, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033-0539, USA
Dr. Jay P. Siegel, FDA, Woodmont II Building, Room 3047 HFM-570, 1401 Rockville Pike, Rockville, MD 20852-1448, USA
Dr. H.L. Spiegelberg, Immunology and Allergy Division, Department of Pediatrics 0609D, University of California, San Diego School of Medicine, San Diego, CA 92093-0609, USA
Dr. John Geigert and Kerri Carter, Immunex Manufacturing Corporation, 51 University Street, Seattle, WA 98101, USA
Dr. William E. Paul and Cynthia Watson, National Institutes of Health, Building 10, Room 11N311, 9000 Rockville Pike, Bethesda, MD 20892, USA
Drs. Monica Tsang and Li Zhou, R & D Systems Inc., 614 McKinley Place NE, Minneapolis, MN 55413, USA
Mr. Louis Westreich, Schering Plough Research Institute, 1011 Morris Avenue (U-2-A), Union, NJ 07083, USA
Dr. Chun C. Chao, Minneapolis Medical Research Foundation, 914 South 8th Street, D3, Minneapolis, MN 55404, USA
Drs. Terry L. Riss and Rich Moravec, Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711-5399, USA

regression (using an in-house program, WRANL (Gaines Das and Tydeman, 1982)) to give an analysis of variance with an assessment of linearity and parallelism of log dose-logit response lines. Estimates of relative potency were obtained as the displacement of parallel log dose-logit response lines.

In a few cases, for which data were insufficient to permit determination of asymptotes, or for which a four parameter logistic function was not a satisfactory description of the dose-response relationship, the available responses from an apparently linear part of the response range were analysed using the customary methods for parallel line assays.

It has been our experience in many assay systems that the within assay replication is not representative of the total assay variation. The collaborative study design thus included two identical ampoules of one IL-4 preparation, coded G and M. The difference between the slopes of these two identical preparations provided an additional measure against which the difference between slopes of non-identical preparations could be assessed. The deviation of the observed ratio of activities of these two identical preparations from the expected value of 1 provided a direct measure of the within assay variability inherent in these systems. The between laboratory variability for estimates of this relative activity, expected to be 1 in all assay systems and laboratories, provided a measure of the minimum variability inherent in this study using these assay systems (inherent study variability, SV).

Estimates of relative potency have been combined as geometric means, and comparisons among them have been made using analysis of variance of the logs of the estimates. Fiducial intervals about mean estimates have been based on the variance of the logs of the estimates combined.

4. Results

4.1. Dose-response relationships

4.1.1. Filler alone

In nearly all assay systems, the excipient preparation gave responses which did not differ significantly from 'control' or zero dose responses and which did not change significantly from one dilution to another and which were therefore omitted from further analysis.

4.1.2. Interleukin-3 (code F) and interleukin-4 (codes G–M)

In the majority of assay systems the log dose-response relationships were adequately described by a four parameter logistic function and the resulting log dose-logit response lines were approximately linear. For some data, estimation of the asymptotes of the four parameter logistic function was either not possible because of limited response ranges or not satis-

Table 3
Numbers and types of assays contributed

Lab. code	Cell line	Measurement of assay[3]	Sensitivity and range (/ml)	Standard used
Cell line proliferation bioassays				
1	CT.h-4S	H-Tdr	10 pg–1 μg	rDNA
3	TF-1	MTT[3]	40 pg–5 ng	*E. coli* rDNA
4	MO7e	H-Tdr	10 pg–2 ng	CHO/yeast rDNA
5	TF-1	MTT[3]	100 ng–1 ng	*E. coli* rDNA
11	CT.h-4S	H-Tdr [3]	1 pg–10 ng	*E. coli* rDNA
13	TF-1	H-Tdr	20 pg–1 ng	*E. coli* rDNA
18	MO7e	MTT[3]	5 ng–80 ng	*E. coli* rDNA
24	CT.h-4S	H-Tdr[3]	7 pg–1 ng	*E. coli* rDNA
27	123	H-Tdr[3]	No cross-react.	Cell supernatant
	CTLL-2	H-Tdr[3]	No cross-react.	Cell supernatant
28	MO7e 251	H-Tdr[3]	25 pg–1 ng	COS cell supernatant
	MO7e 1511	H-Tdr[3]	25 pg–1 ng	COS cell supernatant
29	TF-1	H-Tdr	350 pg–10 ng	Yeast rDNA
32	TF-1	MTT	500 pg–100 pg	None
33	TF-1	MTT	25 pg–10 ng	*E. coli* rDNA
39	CT.h-4S	MTT[14]	2 pg–300 pg	rDNA
44	MO7e	C	1 ng–10 ng	rDNA

Lab. code	Assay format	Sensitivity and range (/ml)	Standard used
Immunoassays			
4	IRMA	50 pg–50 ng	CHO rDNA
6	RIA	15 pg–500 pg	CHO rDNA
7	ELISA	125 pg–2 ng	rDNA
8	ELISA	5 pg–2.5 ng	*E. coli* rDNA
12	ELISA	No data	*E. coli* rDNA
13	ELISA	30 pg–2 ng	*E. coli* rDNA
15	RIA	60 pg–960 pg	*E. coli* rDNA
16	ELISA	100 pg–3 ng	rDNA
17	ELISA	31 pg–2 ng	*E. coli* rDNA
19	IRMA	5 pg–500 pg	CHO rDNA
20	ELISA	2.5 U–10 U	Yeast rDNA
21	ELISA	1 U–50 U	CHO rDNA
22	ELISA	3.5 pg–1 μg	*E. coli* rDNA
25	ELISA	5 pg–1.5 ng	*E. coli* rDNA
26	ELISA	33 pg–33 ng	*E. coli* rDNA
33	ELISA	10 pg–2.5 ng	*E. coli* rDNA
35	ELISA	10 pg–1.25 ng	*E. coli* rDNA
37	ELISA	10 pg–1 ng	CHO rDNA
42	ELISA	30 pg–1 ng	No data
45	ELISA	100 pg–10 ng	*E. coli* rDNA

Lab. code	Assay format	Assay measurement	Sensitivity and range (/ml)	Standard used
Others				
2	Human PBMC	MTT[3]	0.1–10 ng	*E. coli* rDNA
12	T cell lines	H-Tdr[3]	50 pg–1.5 ng	rDNA
13	PHA blasts	H-Tdr	10 pg–5 ng	*E. coli* rDNA
30	B cells	CD23 expression	500 pg 200 ng	*E. coli* rDNA
36	CCL-185	Colonies[3]	10 pg–10 ng	*E. coli* rDNA
41	B cells	H-Tdr	25 pg–100 ng	rDNA
48	RAMOS, G6.C10	CD23 expression	100 pg–2 ng	Yeast rDNA

factory, and analyses were carried out using the linear part of log dose-raw response relationships.

The majority of the cell line bioassays were not specific for either the IL-3 or the IL-4 preparations. Where significant cross-reactivity was seen (TF1 cell line in laboratories 3, 4, 5, 13, 29, 32 and 33 and MO7e cell line in laboratories 4, 18, 28 and 44), the dose levels, and in some cases the form of the dose-response relationships tended to be very different. In these cases the data for the IL-3 preparations were analyzed separately from those for the IL-4 preparations.

Marked anomalies in estimates or within laboratory variances were noted in some assays and in subsequent discussions where deletions of anomalies are noted this will refer to cell line assays in laboratories 11, 12, 28, 30, 41, preparation L, H and in-house standard in some assays from laboratory 13, and immunoassays in laboratories 21, 25, 35, a few assays in laboratory 8 and preparation H in assay 5, laboratory 33.

Data from laboratories 17, 20, 27 and the TF1 cell assays from laboratory 34 and from the IL-4 preparation from laboratory 44 have also been excluded

Table 4
Laboratory geometric mean potency of the various ampouled preparations of IL-4 expressed as international units of 88/656 per ampoule of the ampouled preparation and of the various house standards of IL-4 expressed as nominal ng of 88/656 (assuming one ampoule to contain 100 ng) per 'ng' of house standard

Assay	Laboratory code	88/682 H	89/508 J	89/668 K	92/576 L	In-house Standard
Cell line assays						
CT45_3H	01	246	7696	10896	271	
PBMC_MT	02	770	7911	4912	474	6
TF1_MTT	03	183	4298	7793	616	51
MO7E_3H	04	280	5602	10574	704	72
TF1_3H	04	387	4055	6254	1160	168
TF1_MTT	05	244	9699	9097	518	42
CT45_3H	11 *	169	39786	30806	274	32
T45_3H	12 *	376	8497	5464	590	287
T46_3H	12 *	295	10372	8151	691	234
TK2_3H	12 *	228	7515	5913	517	225
PHA_3H	13	337	4320	2365	518	25
TF1_3H	13	196	6746	9996	760	11
MO7E_MT	18	152	17219	13647	955	
CT45_3H	24	446	5815	7358	655	9
MO7E1_3	28 *	123	40452	80886	1964	
MO7E2_3	28 *	119	12219	6159		346
TF1_3H	29	233	8852	10828	743	
B_LY	30 *	512	19678	9674	950	2011
B_SN	30 *	972	11765	9172	1096	
TF1_MTT	32	677	22741	21741	1223	
TF1_MTT	33	323	8020	9357	513	1827
COL	36	160	0	396	29093	28
BM_Hu4	39	300	8713	6922	353	85
B_3H	41 *	445	12067	9349	479	1144
Ram_CD2	48	520	12968	7937	1007	51000
Geometric mean of laboratory means of immunoassays after the deletions *		321	7874	8508	677	
Interval as % mean		244–423 76–132	5850–10600 74–135	6339–11420 75–134	635–857 79–127	

Table 4 (continued)

Assay	Laboratory code	88/682 H	89/508 J	89/668 K	92/576 L	In-house Standard
Immunoassays						
IRMA4	04	298	10612	10958	602	92
RIA	06	333	8565	6256	371	37
EL4G	07	78	5964	9188	784	232
EL4RD	07	191	8382	6700	458	33
EL4	08	806	2303	1610	109	74
EL4	13	276	15480	6844	532	35
EL4	16	664	8232	3743	524	25
IRMA	19	483	11844	9148	497	64
EL4	21 *	253	10912	21635	580	3054
EL4	22	416	6179	5271	395	62
EL4	25 *	265	13059	14516	727	72
EL4	26	4466	11744	9527	1187	46
EL4	33 *	658	2893	3355	218	15
EL4	35 *	197	499	3800	262	41
EL4	37	593	8964	7195	790	104
EL4	42	576	2458	2434	208	166
EL4	45	466	7981	10980	538	50
Geometric mean of laboratory means of immnoassays after deletions *		428	7161	6316	461	
		256–715	5082–10090	4611–8652	334–637	
Interval as % mean		60–167	71–141	73–137	72–138	
Geometric mean of all laboratory geometric means (95% confidence limits)		339	6457	7507	618	101
		273–422	3701–11265	5812–9697	476–802	54–186
Interval as % mean		80–124	57–174	77–129	77–130	54–185

from further statistical analysis due to lack of species cross-reactivity in laboratories 20 and 27 and non-quantifiable data in laboratories 17, 34 and 44.

4.1.3. Cell line assays and immunoassays

The IL-4 preparations were examined in 23 cell line assay systems. In ten of these systems, no consistent significant differences were detected between the dose-response lines for the IL-4 preparations. In the remaining systems J or both J and K gave different dose-response lines from the other IL-4 preparations; in two of these the dose-response line for L and in two the dose-response line for H was similar to that for J. Under the conditions of laboratory 4, both the TF1 cell line and the MO7e cell line distinguished between the IL-4 preparations with, in the case of the MO7e cells, the dose-reponse line for J being flattest, those for H and K being somewhat flat, those for G and M being steeper and that for L being steepest; in the case of the TF1 cells, the dose-response lines for J and K were flat, those for G and M were intermediate and those for H and L were steep.

The IL-4 preparations were examined in 17 immunoassay systems. In four of these the ampouled preparations were examined for the most part at single dilutions so that dose-response lines were not available for comparison and in five systems no consistent significant differences between the IL-4 preparations were detected. In five of the remaining systems J and K gave different dose-response lines from some or all of the other IL-4 preparations. Preparations G (= M), H or L were also distinguished by some assay systems.

4.1.3.1. Estimates of the relative activity of the IL-4 preparations in terms of one another. Comparisons of each of the ampouled preparations of IL-4 with the interim standard 88/656 (G and M) are shown in Fig. 4 and summarized in Table 4.

For each of these comparisons, estimates of relative activity obtained using immunoassays were at least 10-fold more variable between laboratories than estimates for the coded duplicates, i.e., the inherent study variability (SV).

Except for the comparison of 92/576 (L) with 88/656, the between laboratory variability for estimates from all cell line assays was about twice that of the SV. However, the between laboratory variabilities for estimates from TF1 cell line assays only, comparing 88/682 (H), 89/668 (K) or 92/576 (L) with 88/656, were similar to the SV. For each of these comparisons except 88/682 (H) with 88/656 (G and M), the preparation (J, K or L) appeared to be more biologically active than immunologically active. The ratios of biological to immunological activity, using all cell line assays and all immunoassays were 0.74, 1.60, 1.48 and 1.52 for H, J, K and L respectively.

4.1.3.2. Comparison of IL-4 preparations with the various in-house standards. Comparisons of in-house standards of IL-4 with 88/656 (G and M) are summarized in Table 4. Estimates differed even more widely between assays than did estimates for house standards of IL-3 (between laboratory variability some 30 times the SV for IL-4, some 15 times the SV for IL-3). However, considered overall, there was broad agreement that one ampoule of 88/656 contained 0.1 μg (100 ng) of IL-4.

4.2. Stability

Ampoules of 88/656 which had been stored at elevated temperatures for more than 5 years were compared with ampoules of 88/656 stored continuously at −20°C using three TF1 cell line assays and in two MO7e cell line assays. Results from samples stored at 4°C or 37°C did not differ significantly from those obtained using samples stored continuously at −20°C or from one another. Samples stored at 56°C showed a significant loss of activity which may also reflect greater difficulty in reconstitution after extended storage at this temperature. Failure to detect any loss of activity for samples stored at 4°C or 37°C suggests that there is no significant instability.

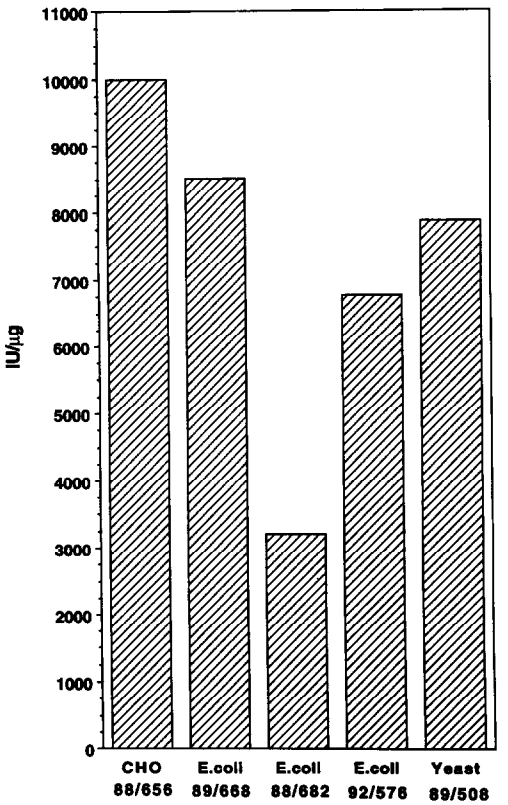


Fig. 1. Relative biological specific activities of recombinant IL-4 preparations included in the study.

5. Discussion

The foremost result generated from the data provided by this study is that in the majority of the assay systems for IL-3 and IL-4 cannot be validly compared with one another and furthermore that the relative activities of pairs of IL-4 preparations may differ depending on the assay system and may not reflect the relative mass contents of ampoules.

This data illustrates a fundamental property of recombinant materials that is not generally taken into account, namely that **recombinant preparations of the same mass may have very different biological specific activities** (Fig. 1).

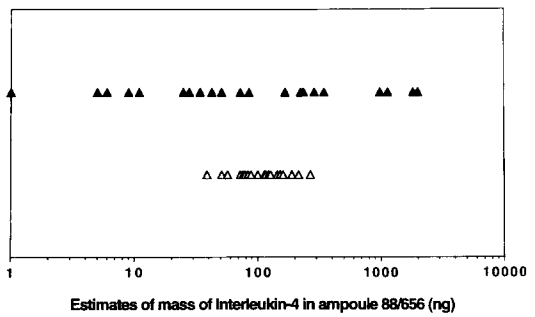


Fig. 2. Relative estimate of the mass of IL-4 in ampoule 88/656 by bioassay using either the various in-house standards of participants (▲) or by comparison with the internal duplicate 88/656 ampoule (△).

This emphasizes the need for a potency standard to be calibrated in biological units and not mass units as these are not equivalent between different recombinant and natural materials.

The need for a single potency standard is evident when comparing the estimates of each preparation to the myriad of in-house standards currently being used. The data in this study illustrate that the inter-laboratory variation using in-house standards is at least 5–10 times that using any of the single ampouled preparations included in the study, i.e., two laboratories can be different by 5–10-fold when estimating the IL-4 content of the same preparation (Fig. 2). The use of a single reference standard substantially decreases this inter-laboratory variation, e.g., the reduction in variation when 89/508, J, is compared with 88/656, G and M, rather than with the in-house standards.

It is also clear that estimates of potency derived from immunoassays consistently disagree with those derived from bioassays. The large differences between estimates from different immunoassay systems, suggest that the specific antibody pairs used in each assay recognise different cytokine preparations to unique and variable degrees (Fig. 3).

The results from this study serve as a reminder that immunoassays may provide precise comparisons of identical preparations or of preparations which are seen to be identical antigenically, but that the antigenic structures recognized by the immunoassay may not reflect biological activity and may not be valid for comparing interleukins derived from different sources (Figs. 1 and 3).

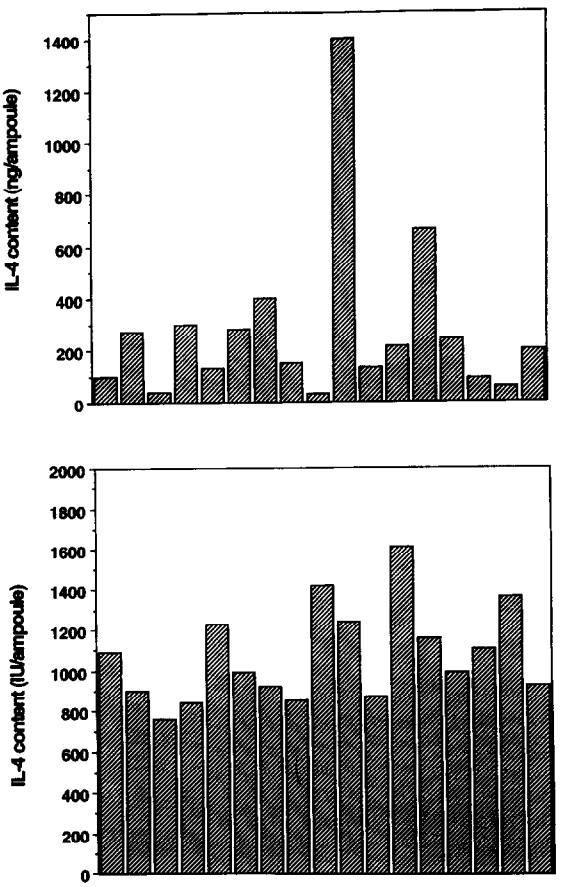
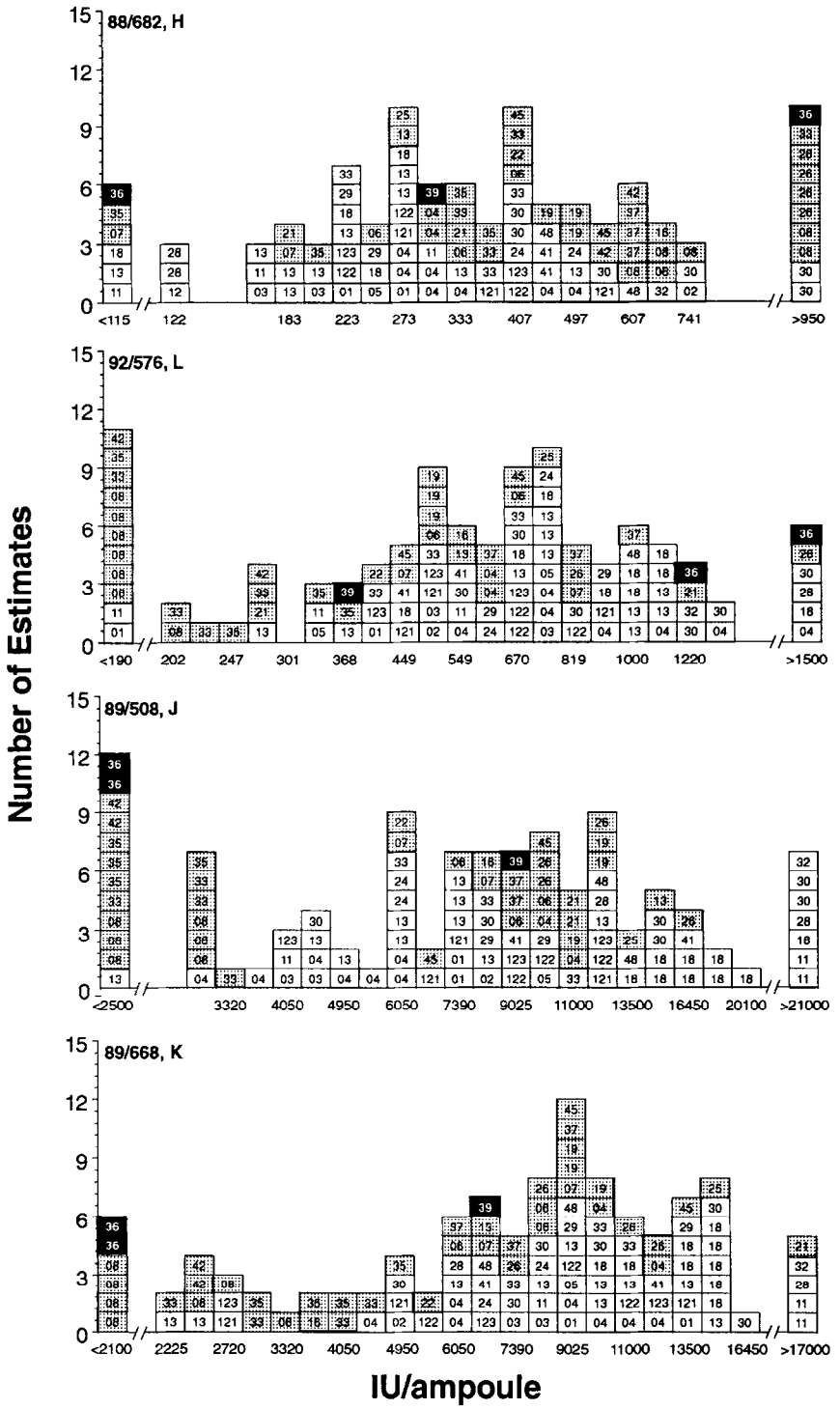


Fig. 3. Immunoassay estimates of IL-4 levels of 88/656 using either the various in-house standards of participants (upper panel) or by comparison with the internal duplicate 88/656 ampoule (lower panel).

The relative activities of the IL-4 preparations were not consistent in all assay systems. Estimates for 89/508, J, compared with 88/656, G and M, or 92/576, L, had larger inter-laboratory variability than other comparisons. The dose-response relations

Fig. 4. Estimates of relative activity of the preparations indicated expressed in international units (IU) of IL-4 in comparison to preparation 88/656. Each box denotes a single assay estimate. Black boxes denote bone marrow assays, stippled boxes immunoassays and plain boxes cell line bioassays.


88/682, H
92/576, L
89/508, J
89/668, K
Number of Estimates
IU/ampoule
<115
122
183
223
273
333
407
497
607
741
>950
<190
202
247
301
368
449
549
670
819
1000
1220
>1500
<2500
3320
4050
4950
6050
7390
9025
11000
13500
16450
20100
>21000
<2100
2225
2720
3320
4050
4950
6050
7390
9025
11000
13500
16450
>17000

for 89/508, J, were also notable for their differences from the other IL-4 preparations (with the possible exception of 89/668, K in some systems). It is also noted that 89/508, J, is the only IL-4 preparation to have been mutated in specific regions, although it is not known if this accounts for the observed differences. There were no apparent consistent differences observed among the other preparations. The IL-4 preparation 88/656, G and M, appears equally suitable to any of the other preparations, with the possible exception of 89/508, J, to serve as an international standard, and no problems have been reported with its use as an interim standard since 1988. It was therefore agreed that 88/656 be established as the international standard for IL-4 and that it be assigned the unitage of 1000 IU/ampoule (the unitage adopted for its use as an interim standard) (Fig. 4).

6. Conclusions

The data contributed to this study illustrate the need for a standard for IL-4 calibrated in specific biological potency units. The results of this study also provide confirmation of the accuracy and precision of bioassays used for calibration purposes and illustrate the possible difficulties in calibration which may arise if the differences in biological and immunological activities are ignored.

Based on the results of this study the preparation in ampoules coded 88/656 was established by the WHO Expert Committee on Biological Standardization (ECBS) as the international standard for IL-4 with an assigned potency of 1000 IU. There are approximately 3400 ampoules of 88/656 available for distribution. These materials are stored at the National Institute for Biological Standards and Control and are available for world-wide distribution.

Acknowledgements

We thank Deborah Richards for processing this report. We acknowledge Gill Creeber for data processing and the Standards Processing Division, in particular Dr. Peter Dawson, for the preparation of the ampouled materials. We would also like to thank all the participants of this study and the companies who generously donated materials – Schering Plough Corporation, USA; Immunex Corporation, USA; Sandoz, Switzerland; Glaxo, UK; and Innogenetics, Belgium.

References

Avanzi, G.C., Lista, P., Giovinazzo, B., Miniero, R., Saglio, G., Benetton, G., Coda, R., Cattoretti, G. and Pegoraro, L. (1988) Selective growth response to IL-3 of a human leukaemic cell line with megakaryoblastic features. Br. J. Haematol. 69, 356.

Defrance, T., Fluckiger, A.C., Rossi, J.F., Magaud, J.P., Sotto, J.J. and Banchereau, J. (1992) Antiproliferative effects of interleukin-4 on freshly isolated non-Hodgkin malignant B-lymphoma cells. Blood 79, 990.

Gilleece, M.H., Scarffe, J.H., Ghosh, A., Heyworth, C.M., Bonnem, E., Testa, N., Stern, P. and Dexter, T.M. (1992) Recombinant human interleukin-4 (IL-4) given as daily subcutaneous injections – A phase I dose toxicity trial. Br. J. Cancer 66, 204.

Golumbek, P.T., Lazenby, A.J., Levitsky, H.I., Jaffee, L.M., Karasuyama, H., Baker, M. and Pardoll, D.M. (1991) Treatment of established renal cancer by tumour cells engineered to secrete interleukin-4. Science 254, 713.

Howard, M., Farrar, J., Hilfiker, M., Johnson, B., Takatsu, K., Hamaoka, T. and Paul, W.E. (1982) Identification of a T cell derived B cell growth factor distinct from interleukin 2. J. Exp. Med. 155, 914.

Margolin, K., Aronson, F.R., Sznol, M., Atkins, M.B., Gucalp, R., Fisher, R.I., Sunderland, M., Doroshow, J.H., Ernest, M.L. and Mier, J.W. (1994) Phase II studies of recombinant human interleukin-4 in advanced renal cancer and malignant melanoma. J. Immunother. Emphasis Tumor Immunol. 15, 147.

Ohara, J., Coligan, J., Zoon, K., Maloy, W.L. and Paul, W.E. (1987) High-efficiency purification and chemical characterization of B cell stimulatory factor-1/interleukin-4. J. Immunol. 139, 1127.

Ohara, J. (1988) Interleukin-4: Molecular structure and biochemical characteristics, biological function, and receptor expression. Year Immunol. 5, 126.

Siegel, J.P. and Mostowski, H.S. (1990) A bioassay for the measurement of human interleukin-4. J. Immunol. Methods 132, 287.

Toi, M., Bicknell, R. and Harris, A.L. (1992) Inhibition of colon and breast carcinoma cell growth by interleukin-4. Cancer Res. 52, 275.

Topp, M.S., Koenigsmann, M., Mire-Sluis, A., Oberberg, D., Eitelbach, F., Marschall, Z. von, Notter, M., Reufi, B., Stein, H., Thiel, E. and Berdel, W.E. (1993) Recombinant human interleukin-4 inhibits growth of some human lung tumor cell lines in vitro and in vivo. Blood 82, 2837.

Yokota, T., Otsuka, T., Mosmann, T., Banchereau, J., Defrance,

T., Blanchard, D., de Vries, J.E., Lee, F. and Arai, K. (1986) Isolation and characterization of a human interleukin cDNA clone, homologous to mouse B-cell stimulatory factor-1, that expresses B-cell- and T-cell-stimulatory activities. Proc. Natl. Acad. Sci. USA 83, 5894.

Yokota, T., Arai, N., DeVries, J., Spitz, H., Banchereau, J., Zlotnick, A., Rennick, D., Howard, M., Takebe, Y., Miyatakes, L.F. and Arai, F. (1988) Molecular biology of interleukin-4 and interleukin-5 genes and biology of the product that stimulate B cells, T cells and hematopoietic cells. Immunol. Rev. 102, 172.