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Idiopathic paraproteinaemia

I. STUDIES IN AN ANIMAL MODEL—THE AGEING C57BL/KaLwRij MOUSE

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SUMMARY

A search for a suitable animal model for studies on idiopathic paraproteinaemia showed that an age-dependent increase in the appearance of homogeneous immunoglobulins in serum was common to all of the seven mouse strains investigated to date. The highest frequency was found in C57Bl/KaLwRij mice. Further investigations in this strain demonstrated that, except for some quantitative differences, most of the features of human and C57Bl mouse idiopathic paraproteinaemia were essentially the same. No clear-cut correlation was found between the idiopathic paraproteinaemia and, in the old C57Bl mice, a rather frequently occurring reticulum cell sarcoma B and amyloidosis. The mouse idiopathic paraproteinaemia can be regarded as an analogue of the human idiopathic paraproteinaemia and therefore as a suitable model for further experimental studies.

INTRODUCTION

An increasing frequency of homogeneous immunoglobulins (H-Ig)—paraproteins—in the sera of ageing humans without a B-cell malignancy has been repeatedly observed (Hällen, 1966; Zawadski & Edwards, 1972; Waldenström, 1973; Kyle & Bayrd, 1976). Several names have been given to this condition, but it is most often designated as benign monoclonal gammapathy or idiopathic paraproteinaemia (IP). Clinical and laboratory studies in man have revealed that IP occurs in about 1% of the adult population. There is a clear age-related increase from 0% in the third decade up to 19% in the tenth decade of life (Axelsson, Bachman & Hällen, 1966; Englisova *et al.*, 1968; Radl *et al.*, 1975a). IP is regarded as essentially 'benign'; development into myeloma or Waldenström's macroglobulinaemia was observed in only a few cases, despite the large number of cases of IP observed over long periods. From the literature cited above it can be calculated that IP occurs about 100 times more frequently than its malignant counterpart, i.e., paraproteinaemia due to a B-cell malignancy. In the majority of cases, IP is characterized by a constant serum level of the paraprotein, usually below 2g/100ml. This level is generally maintained over many years, often till the individual's death. It is mainly this feature that distinguishes IP from other conditions accompanied by a production of H–Ig (Radl, 1976). The majority of the Ig belong to the IgG class (±60%). Paraproteins of the IgM and the IgA class are represented by approximately equal proportions (±20%). IP of IgD class, or an appreciable amount of Bence Jones protein, was observed only in exceptional cases. Immunoglobulin levels of classes other than that of the paraprotein are usually normal or slightly decreased (Radl *et al.*, 1975a). Antibody activity of the paraproteins was established in only a small number of cases (Seligmann & Brouet, 1973; Potter, 1973a). There are indications that genetic factors may play a role in the development of IP (Meijers, De Leeuw & Voormolen-Kalova, 1972; van Camp, Cole & Peetermans, 1977).

The aetiology, mechanisms and significance of this particular form of immunoglobulin production in

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ageing humans remain unknown. For their elucidation studies with an animal model should be useful; however, findings have not been reported in animals until recently (Radl & Hollander, 1974). Our studies performed on ageing C57Bl mice show that the mouse IP can be regarded as an analogue of the human IP and therefore as a suitable model for further experimental studies.

MATERIALS AND METHODS

Mice. C57Bl/KaLwRij, BALB/c, CBA/Rij, C3H, NZB, F_1(C57Bl/Rij × DBA/2), and F_1(C57Bl/Ka × CBA/Rij) mice from colonies at the Institute for Experimental Gerontology TNO were investigated. They were maintained under conventional conditions and were allowed to live out their life spans. Blood from the tail vein was collected at 3 month intervals. Necropsies and histological examinations were performed according to a standard protocol.

Methods. Investigation for the presence of H-Ig in sera was performed by agar electrophoresis according to Wieme (1959), by immunoelectrophoresis and by immunofixation (Cejka & Kithier, 1976), using polyvalent and monospecific antisera. Antisera to IgM, IgA, IgG1, IgG2b and Fab fragment of IgG were prepared as described previously (Bloemmen *et al.*, 1976). Sera against IgG3 and λ light chains were raised in sheep, goats and rabbits in a similar way, using purified myeloma proteins J606, FLOPC 21 and RPC 20. Mice, bearing these tumours were a generous gift of Dr M. Potter, NIH, Bethesda, Maryland, USA. Antisera to IgG2a were prepared by immunization of sheep and goats with different IgG2a paraproteins of C57BL mice. After absorption, these antisera mainly recognised the allotypic (Ig1b) and to a lesser extent the subclass specific determinants of the IgG2a. Antiserum specific for κ light chains was prepared from the anti-Fab serum by absorptions over immunoadsorbents consisting of insolubilized purified IgM-λ (MOPC 104 E), IgG2a-λ (HOPC-1) and IgA-λ (MOPC 315) paraproteins. Antisera specific for idiotypic determinants of various H-Ig of the C57BL mice were raised in guinea-pigs according to a technique described previously (Radl, de Glopper & de Groot, 1978).

The levels of individual Ig classes and subclasses were determined by the Mancini single radial immunodiffusion technique in a modification described by Voormolen-Kalova, Van den Berg & Radl (1974). The concentration of some of the paraproteins was estimated by scanning densitometry of the electrophoretic plates and by determining the proteinaemia by the biuret method. Screening for Bence Jones proteins was performed in up to fifty times concentrated urine samples by agar electrophoresis and immunoelectrophoresis. Screening for antibody activity of the paraproteins in the sera of the C57Bl mice was performed by the double radial diffusion technique in Ouchterlony plates containing 3% polyethylene glycol 6000 (PEG). Sera were tested against a set of the following antigens: 5-Acetyluracil-1-BSA; purine-6-BSA; adenosine-5-monophosphate-BSA; uridine-5-monophosphate-BSA; cytosine-BSA; guanosine-BSA; p-azo-benzenearsenate-BSA; D-penicillamine-HSA; 2,4-dinitrophenyl-BSA; 2,4-dinitrophenyl-MoSA; 2,4-dinitrophenyl-ovalbumin; phosphorylcholine-BSA; B 1355 dextran; pneumococcus C polysaccharide III; streptococcal group A and C polysaccharides; lipopolysaccharides of *Salmonella typhimurium*, Citrobacter 396, *E.coli* 08, *E.coli* 058, *E.coli* 0100, *E.coli* 0111, *E.coli* 0124, and capsular polysaccharides of *E.coli* K27, K42, K85 and K87; and heterogeneous IgG from man, the mouse, the rat, the guinea-pig and the rabbit. In addition, sera from some mice over 20 months of age which were infested with mites, were tested against soluble extracts of these parasites.

RESULTS

When screening the sera of mice of seven different strains, H-Ig were found in ageing individuals in each (Fig. 1). However, the onset, frequency and class expression of the H-Ig in each of the strains were different. Nearly all of the H-Ig in the NZB and C3H mice were of the IgM class only. All Ig classes and subclasses were represented among the H-Ig in mice of the other strains. While the CBA and BALB/c mice developed H-Ig at low frequency and only at the end of their life, the C57Bl mice already showed some H-Ig in their sera at the age of 3 months and their frequency markedly increased throughout their life span. The incidence of H-Ig in the C57Bl mice was higher in females than in males. The 90%, 50% and 10% survival values for males were, respectively, 14, 24 and 28 months; for females, they were 15, 22 and 27 months. The frequency curve for H-Ig in the F_1(C57Bl × DBA) mice showed a later onset and a steep increase in the older animals. Preliminary data for the F_1(C57Bl × CBA) mice showed a curve between those of the C57Bl and the CBA mice.

These data give percentages of mice with H-Ig within the given age groups, regardless of the origin of the H-Ig. If we assume that conditions accompanied by a production of H-Ig in mice are similar to those in man (Radl, 1976), it would indicate that not all H-Ig reflect an IP. Some could be products of B-cell malignancy; others might be transient homogeneous antibodies. Follow-up studies and histological examinations of dead animals of the CBA, BALB/c and C57Bl strains excluded both these conditions as a source of H-Ig production in the majority of the cases.

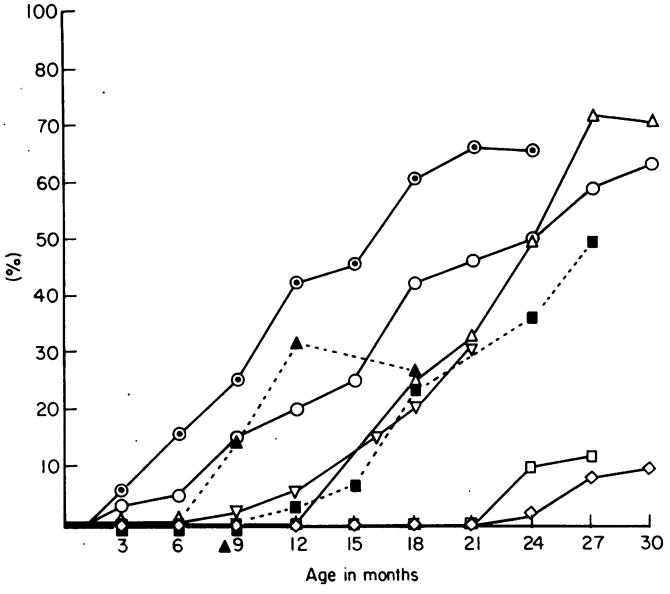

FIG. 1. Frequency of homogeneous immunoglobulins in the sera of mice of different strains during ageing. (○———○) C57Bl ♂ ($n = 465$); (△———△) F_1(C57Bl × DBA) ♀ ($n = 176$); (□———□) BALB/c ♂ ($n = 327$); (◇———◇) CBA ♂ ♀ ($n = 359$); (⊙———⊙) C57Bl ♀ ($n = 302$); (▽———▽) F_1(C57Bl × CBA) ♂ ♀ ($n = 272$); (▲----▲) NZB ♂ ♀ (IgM) ($n = 124$); (■---■) C3H ♂ ♀ (IgM) ($n = 204$).

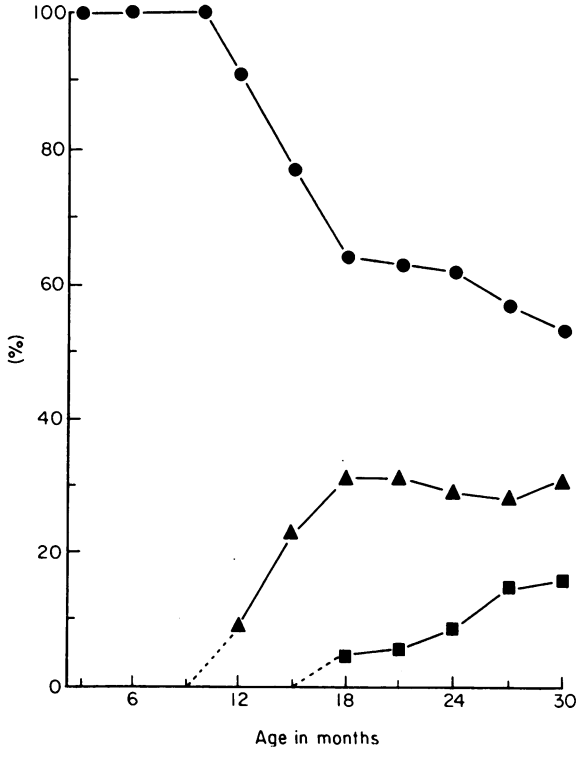

FIG. 2. Percentage distribution of single (●), double (▲) and triple (■) paraproteins in sera of the ageing C57Bl/KaLwRij mice ($n = 451$).

Because of the very high frequency of H-Ig in the C57Bl mice, this strain was chosen for further studies in order to determine to what extent this mouse paraproteinaemia can be regarded as an analogue of the human IP. Distribution of Ig classes and subclasses among the paraproteins was determined in sera from 243 mice with a persistent paraproteinaemia. The percentage distribution for IgA, IgM, IgG1, IgG2a, IgG2b, and IgG3 was 1·2, 12·8, 22·6, 38·2, 19·0 and 6·2, respectively. The analysis of the light chain type of 110 paraproteins showed that only ten of the paraproteins belonged to the λ type. Of these, five were IgM, two were IgG1, two were IgG3 and one was IgG2b.

TABLE 1. Frequency of different combinations of Ig classes and subclasses in multiple paraproteinaemias in the C57Bl mice (n = 100)

Paraproteins	Percentage
IgG2a + IgG1	26
IgG2a + IgG2b	21
IgG2a + IgM	12
IgG2a + IgG1 + IgG2b	8
IgG1 + IgM	7
IgG1 + IgG2b	7
IgG2a + IgG1 + IgM	4
IgG2a + IgG3	3
IgG2a + IgG3 + IgM	3
IgG2a + IgG2b + IgG3	2
IgG2a + IgG2b + IgM	2
IgG3 + IgM	1
IgG2b + IgM	1
IgG2a + IgG2a	1
IgG1 + IgG2b + IgM	1
IgG2a + IgG2b + IgG1 + IgG3	1
Total	100

With the increasing age of the animals, the number of multiple paraproteinaemias also increased. The percentage distribution of single, double and triple paraproteins (a total of 451) in sera of ageing C57Bl mice is shown in Fig. 2. Analysis of the frequency of different paraprotein combinations in these sera showed that here too the IgG2a subclass was most often represented (Table 1). Bence Jones protein was not found in the urine of fifty animals with IP. It was, however, detected in the urine of two mice suffering from lymphosarcoma associated with an IgM paraproteinaemia.

The follow-up studies in 220 mice with a paraproteinaemia showed that only a small minority of the paraproteins (10%) were transient in their appearance. The possibility of a paraproteinaemia disappearing was higher either during the first year or at the end of the animal's life. In the latter case, the other Ig often decreased also, sometimes a new paraprotein appeared and, at post-mortem, a reticulum cell sarcoma type B (according to Dunn's classification, 1954) was found. In the majority of cases once paraproteins developed they remained at about the same concentration (usually below 4mg/ml) for the rest of the individual's life.

The influence of the paraproteinaemia on the levels of Ig of other classes and subclasses was studied in 27 month old mice. The differences between the Ig levels of two groups (twenty-two mice with and sixteen mice without a paraproteinaemia) were analysed by the Student's t-test. Only individual Ig classes and subclasses which did not contain a paraprotein were analysed in the group of mice with a paraproteinaemia. No significant difference was found between the two groups for Ig of any class or subclass.

Tests for antibody activity of 300 paraproteins were performed in PEG Ouchterlony plates against

TABLE 2. Correlation between lymphoreticular malignancy and paraproteinaemia in C57BL mice, age over 15 months

Malignancy	Paraproteinaemia	Number of cases
Lymphosarcoma	+	2
	−	0
Reticulum cell sarcoma	+	13
	−	9
None	+	48
	−	28
Total		100

Total number of mice with lymphoreticular malignancy = 24%.

thirty-five different antigens. Only two sera gave a positive reaction. One serum containing an IgG2a paraprotein precipitated B 1355 dextran. The other with IgG2a, IgG2b and IgG3 paraproteins reacted with 5-acetyluracil-BSA, DNP-BSA, DNP-MoSA, DNP-ovalbumin and phosphorylcholine-BSA. Immunoelectrophoretic investigation showed that the IgG3 paraprotein was responsible for reaction with the DNP-BSA antigen. Because of the small amounts of sera which were available from individual mice, no further detailed examinations could be performed.

TABLE 3. Comparison between idiopathic paraproteinaemia (IP) in man and the mouse

Idiopathic paraproteinaemia	Human	C57Bl/KaLwRij
Frequency increases with age	Up to 19% in 10th decade	Up to 60% at 30 months (♂)
Essentially 'benign'	Yes	Yes
Frequency of IP/malignant paraproteinaemia	About 100/1	Probably > 100/1
Persistent at a steady level	Yes	Yes
Usually during rest of life	Yes	Yes
Serum paraprotein concentration	About < 2g%	< 0·4g
Criteria for monoclonal Ig fulfilled	Yes	Yes
Class distribution of paraprotein	IgG ≫ IgM ≅ IgA	IgG ≫ IgM > IgA
Excess production of Bence Jones protein	Exceptionally	Not yet found
Number of paraproteins	Usually 1	Often > 1
Level of other Ig	Normal or slightly decreased	Normal
Antibody activity of IP known	Exceptionally	2 out of 300 (tested against 35 different antigens)
Genetic predisposition	Probably	Most likely

Antisera specific for idiotypic determinants were prepared against twenty-two different paraproteins. These antisera recognized only their homologous paraproteins and gave negative reactions when tested with 50 other paraproteins. Only one antiserum (C6, against an IgG2a paraprotein) cross-reacted with one other (IgM) paraprotein. However, an antiserum (C42) against the IgM paraprotein idiotype did not precipitate the C6 IgG2a paraprotein. Small amounts of individual mouse sera were also a limiting factor for further studies here.

Results of detailed studies on the pathology and immunopathology of the C57Bl/KaLwRij strain will be published elsewhere (Zurcher *et al.*, in preparation). Here, only data relevant for the differential diagnosis between IP and paraproteinaemia of a B-cell malignancy will be given. Results from 100 autopsies showed a rather high incidence of lymphoreticular malignancies in the ageing C57Bl mice

(Table 2). While both cases of lymphosarcoma were accompanied by a paraproteinaemia (IgM class), there was no great difference in the number of mice with or without a paraproteinaemia in the group suffering from reticulum cell sarcoma B. Our preliminary investigations do not yet enable us to classify properly the malignant cell in the C57Bl reticulum cell sarcoma B. However, malignant spleen cells from a number of mice with an advanced reticulum cell sarcoma B did not contain Ig in their cytoplasm or on the membrane, as tested by immunofluorescence. They were also not recognized by a conjugate specific for T-cell markers (Nordic Immunological Laboratories, Tilburg, The Netherlands). Other data support the idea that the vast majority of the H-Ig in the ageing C57Bl mice were IP and not a product of a lymphoreticular malignancy. Longitudinal studies revealed that H-Ig began to appear as early as 3 months of age in some animals and this had no obvious negative influence on the life expectancy of those mice, many of which did not develop a malignancy. In many of the cases with reticulum cell sarcoma B, the level of all Ig decreased and the long lasting single or multiple paraproteinaemia disappeared in the terminal period. In addition, follow-up studies and transplantation experiments in mice with a B-cell lymphoma or a spontaneous diffuse myeloma demonstrated a different behaviour of these malignant paraproteinaemias as compared to the IP (Radl *et al.*, in preparation). In this stage of our investigations, it is very difficult to estimate exactly the ratio between IP and paraproteinaemias of a B-cell malignancy but it may be higher than 100 : 1.

The combined results which are pertinent to the comparison between IP in man and mouse are summarized briefly in Table 3.

DISCUSSION

The search for a suitable animal model for studies on IP showed that an age-dependent increase in the appearance of H-Ig in serum was common to all mouse strains investigated so far. However, the highest frequency of H-Ig was found in the C57Bl/KaLwRij mice. Therefore, further investigations were directed to this strain in order to determine whether the mouse IP can be regarded as an analogue of the human IP.

Results of this study showed that, except for some quantitative differences, most of the features of human and C57Bl mouse IP are essentially the same. Some of the quantitative differences could be readily explained. For example, the lower levels of paraproteins (and also of other Ig) in mice may reflect the higher catabolic rate of mouse Ig (Spiegelberg, 1974). The much higher frequency of IP in the C57Bl mice, as compared to the human IP, may be the result of differences between the highly inbred and outbred populations. A somewhat surprising finding was the relatively low frequency of IgA paraproteins in the C57Bl mice. This may reflect differences in the IgA system between man and mouse. While the secretory and the systemic IgA systems in man produce IgA of a different form (the secretory system mainly producing polymeric IgA, the systemic mainly monomer IgA), such a difference was not observed in mice (Tomasi & Grey, 1972; Radl, Swart & Mestecky, 1975b).

On the other hand, the rare finding of an IgA IP in the C57Bl mice may be due to technical problems related to the detection of an IgA paraprotein at a low concentration. Its relatively fast anodic mobility very often excludes the usefulness of agar electrophoresis, because of the presence of other non-Ig protein bands with a comparable electrophoretic mobility. Investigation by immunoelectrophoresis and immunofixation may also have a limited discrimination value if the C57Bl IgA paraproteins behave as do many of the known IgA paraproteins from plasma cell tumour bearing BALB/c mice. They often show broad bands in the immunofixation plates and assymetric precipitin lines in immunoelectrophoresis. In addition, the light chain typing seems to be little help because no λ type IgA was detected among the 110 paraproteinaemic sera tested so far. These problems may mean that only a fraction of the IgA paraproteins were recognized. This question has still to be studied further.

Some other features which deserve more attention were revealed in the animal model. Firstly, the incidence of IP in C57Bl mice was higher in females than in males. A survey of published case reports of human IP may indicate that both the survival and the incidence of IP is the reverse of the situation in mice, as far as sex is concerned.

The rather high incidence of reticulum cell sarcoma type B in the C57Bl strain raises the question of a possible relationship between IP and this malignancy. Several problems connected with this question were reviewed and discussed in greater detail by Potter (1972 and 1973b) and by Warner, Potter & Metcalf (1974). A somewhat different morphology and the properties of this tumour type in different mouse strains may indicate a heterogeneity, as far as the underlying neoplastic cell type is concerned. Our results indicate that the IP cannot be a direct consequence of this malignancy; it cannot be excluded however, that both conditions stem from the same basic defect in the immune system in this mouse strain. There is no published information documenting a higher incidence of lymphoreticular malignancies in humans with IP. Only children suffering from some immunodeficiency diseases, especially the Wiskott–Aldrich syndrome, have a high incidence of lymphoreticular malignancies and paraproteinaemias (Kersey, Spector & Good, 1975; Radl *et al.*, 1976). In such cases, however, the paraproteins were usually transient. In this connection, reports on the higher incidence of IP in relatives of myeloma or Waldenström's macroglobulinaemia patients should also be noted (Meijers *et al.*, 1972).

Another point of interest is the finding of a high incidence of amyloid in old C57Bl mice (males 83%, females 60%) (Zurcher *et al.*, in preparation). So far, no clear-cut correlation has been found between the incidence of IP and amyloidosis in the C57Bl mice. While deposits of amyloid are frequently found in aged humans and in certain human B-cell malignancies, no explicit data are available on amyloidosis in individuals with an IP. This subject will be further elaborated in a separate study.

Summarizing the information available and bearing in mind all the differences mentioned, it seems clear that the C57Bl/KaLwRij mouse can be considered as a useful model for studying idiopathic paraproteinaemia—an unexplained phenomenon of ageing.

ADDENDUM

Sera from two C57Bl mice gave a positive reaction with anti-mouse IgD serum (Sera-Lab, Sussex, England) when tested in Ouchterlony plates. Using the same antiserum in immunoelectrophoresis, both of these sera showed a symmetrical precipitin line in the $\gamma 1$ region, corresponding to a weak homogeneous component found by agar electrophoresis. This component could not be identified previously by any other antiserum. The anti-mouse IgD serum reacted neither with several pools of normal mouse sera nor with 240 paraproteins.

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