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The influence of genetic factors associated with the immunoglobulin heavy chain locus on the development of benign monoclonal gammapathy in ageing Igh-congenic mice

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SUMMARY

The role of genetic factors associated with the immunoglobulin heavy chain locus (Igh) in the development of benign monoclonal gammapathy (BMG), a benign B-cell proliferative disorder, was investigated in six Igh congenic mouse strains during ageing. The strains used had a C57BL or BALB background: C57BL/6, BALB.Ig^b and CB-20 carrying the C57BL Igh (Igh^b allotype), BALB/c and C57BL/6.Ig^a carrying the BALB/c Igh (Igh^a allotype) and BAB-14, that is of BALB/c origin with the exception of the constant part of the Igh, which is of C57BL origin. The frequency of homogeneous immunoglobulins (H-Ig), both single and multiple, was the highest in C57BL/6 mice, followed by C57BL/6.Ig^a. The frequencies of H-Ig in BALB.Ig^b and CB-20 mice were higher than those of BALB/c and BAB-14, although somewhat lower than in C57BL/6.Ig^a mice. Multiple H-Ig were found especially in the sera of C57BL/6 mice. Categorization of the monoclonal gammapathies (MG) on the basis of their origin showed a single transient monoclonal B-cell proliferation in 0–8% of the mice of all strains. Persistent, non-progressive MG, presumably BMG, were detected in 64% of C57BL/6, 30% of C57BL/6.Ig^a, 22% of BALB.Ig^b, 17% of CB-20, 13% of BAB-14 and 6% of BALB/c mice. Multiple myeloma or Waldenström-like B-cell lymphoma were found to be responsible for 2–4% of the paraproteinemias in all strains. The remaining H-Ig, varying from 11% of the C57BL/6 to 70% of the BAB-14 mice, could not be evaluated in time. The most frequent isotypes of the BMG within C57BL/6 and C57BL/6.Ig^a were IgG2^a and IgG2^b, respectively; IgM was the most frequent isotype within the four BALB congenic strains. The immunoglobulin heavy chain allotypes under investigation appeared to be only partly related to the onset, occurrence, multiplicity and persistence of the BMG developing in these Igh congenic C57BL and BALB strains during ageing. The immunoglobulin heavy chain allotypes, however, were not related to the major isotype of the BMG. The results obtained in CB-20 and BALB.Ig^b on the one hand, and in BAB-14 on the other hand, may suggest a role for the variable part of the Igh in the development of BMG. Since no absolute influence could be ascribed to the Igh, we assume that primarily other genetic sequences regulating proliferative B-cell functions account for the pathogenesis of BMG.

INTRODUCTION

Benign monoclonal gammapathy (BMG) is the result of an irreversible intrinsic defect within a single B-cell clone (Radl, 1982). Factors extrinsic to the affected B-cell clone, such as a T-immune system deficiency and chronic and excessive antigenic stimulation, contribute to the development of BMG in its early stages (Radl, 1979; Van den Akker, Brondijk & Radl, 1988; Van den Akker *et al.*, 1988). A strong indication for genetic influences in the development of BMG has been obtained by the finding of various frequencies of homogeneous immunoglobulins (H-Ig) in ageing mice of different mouse strains (Radl *et al.*, 1978). In that study, C57BL mice showed a high frequency and early onset of BMG in contrast to CBA and BALB/c mice, in which a low frequency and late onset of H-Ig was observed. Further support for the influence of genetic factors on the pathogenesis of BMG was obtained in experiments using radiation chimeras of the C57BL and CBA strains (Radl *et al.*, 1984). Considering the genes which could be candidates for possible pathogenetic factors in the occurrence of monoclonal gammapathies (MG), the major histocompatibility complex (MHC) and the immunoglobulin heavy chain locus (Igh) appeared among the most obvious. However, no correlation

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Table 1. Origin and Igh characteristics of the congenic mouse strains used

Strain	Inbred background	Strain source of introduced Igh	IgC*	IgV†
C57BL/6J	C57BL/6J	—	b	b
C57BL/6.Ig^a	C57BL/6	BALB/c	a	a
CB-20	BALB/c	C57BL/Ka	b	b
BALB.Ig^b	BALB/c	C57BL/6	b	b
BAB-14	BALB/c	C57BL/Ka	b	a
BALB/c	BALB/c	—	a	a

Modified according to R. Lieberman (1978).
*IgC, constant part of Igh.
†IgV, variable part of Igh.

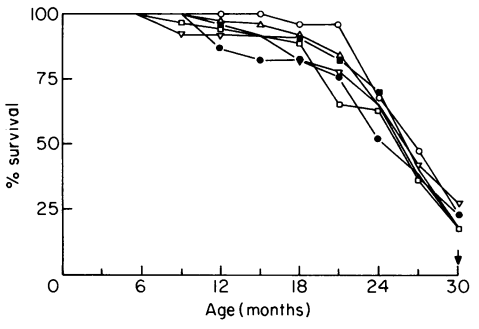

Figure 1. Survival of the Igh congenic C57BL and BALB mice used. (●), C57BL/6; (■), C57BL/6.Ig^a; (△), CB-20; (□), BALB.Ig^b; (▽), BAB-14; (○), BALB/c. Arrow indicates termination of the study at 30 months of age.

between the H-2 haplotype and BMG could be determined (Van den Akker *et al.*, 1987). With regard to the Igh, we recently found that within the F_1 generation of the low BMG frequency strain CBA and the high BMG frequency strain C57BL, the H-Ig of the IgG2^a and IgD classes which developed with ageing carried predominantly the *b* allotype of the C57BL strain (Radl *et al.*, 1985c). These data suggested a role of Igh-associated genetic material from the parental C57BL strain in the development of BMG in the ageing F_1 mice.

In the present study, the influence of the Igh in the development of MG was investigated in six ageing Igh congenic C57BL and BALB strains. A partial correlation between a high frequency of MG and the presence of the *b* (C57BL) allotype of the Igh was found. However, the influence of the Igh on the pathogenesis of BMG was not found to be a dominant one.

MATERIALS AND METHODS

Mice

BALB/c and C57BL/6 mice were purchased from Olac Ltd, Bicester, Oxon, U.K. The BALB.Ig^b breeding stocks were obtained from Professor E. Kölsch, Department of Immunology, University of Münster, Münster, FRG. The CB-20, BAB-14 and C57BL/6.Ig^a breeding stocks were obtained from the Basel Institute for Immunology, Basel, Switzerland. The BALB.Ig^b, CB-20, BAB-14 and C57BL/6.Ig^a mice were bred in our own department. The C57BL/6.Ig^a strain is Igh congenic to the original C57BL/6 (Igh^b) strain. CB-20 and BALB.Ig^b are Igh congenic to the original BALB/c (Igh^a) strain. The BAB-14 strain is fully syngeneic to BALB/c except for the constant part of the Igh which is of C57BL origin. A survey of the mouse strains used and their Igh characteristics is presented in Table 1. Samples of the sera of all mouse strains were taken and tested initially for the presence of the correct Igh product. All strains were found to have the right Igh-allotype. The C57BL/6, C57BL/6.Ig^a, CB-20, BALB.Ig^b, BAB-14 and BALB/c groups consisted of 53, 83, 101, 100, 86 and 50 mice, respectively. All mice were maintained under clean, conventional conditions, where they were allowed to live out their life-spans. The mice received non-autoclaved diet AM II (Hope Farms, Woerden) and acidified water (pH 3·5) *ad libitum*. Blood samples were taken at 3-month intervals and the serum was stored frozen at −20° for later examination. In order to obtain sufficient

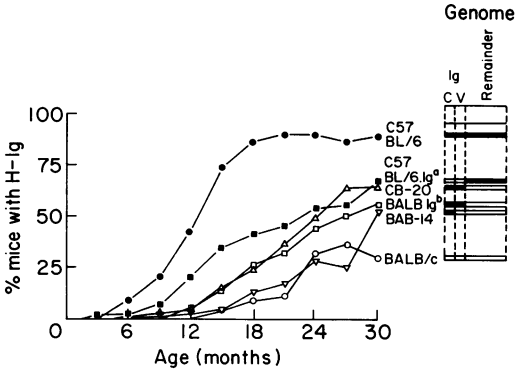

Figure 2. Frequency of H-Ig in the sera of Igh congenic C57BL and BALB mice during ageing. (●), C57BL/6; (■), C57BL/6.Ig^a; (△), CB-20; (□), BALB.Ig^b; (▽), BAB-14; (○), BALB/c. On the genome chart the genetic make-up of each mouse strain is represented by bars next to the frequency curve of that particular strain. IgC, constant part of Igh, IgV, variable part of Igh; remainder, entire genome without Igh; A black bar indicates C57BL origin, a white bar indicates BALB/c origin.

material of the different mouse strains for histopathological examination, the experiments were finished at 30 months of age, when about 20% of all mice were still alive.

Detection of homogeneous Ig components

Sera were investigated for the presence of H-Ig by agar electrophoresis according to Wieme and by immunofixation performed on Wieme's agar plates (Radl, 1981) using a goat antiserum against all mouse Ig. Sera with a H-Ig component were investigated further by immunoelectrophoresis using goat or sheep antisera specific for mouse IgM, IgG1, IgG2a, IgG2b, IgG3, IgA and lambda light chain isotypes (Radl, 1981). All polyclonal sheep, goat and rabbit antisera were prepared at the TNO Institute for Experimental Gerontology, Rijswijk, as described earlier (Radl, 1981).

Mice were considered as positive for H-Ig, and therefore included in the calculations, when serum analysis by combination of agar electrophoresis, immunoelectrophoresis and immunofixation revealed a H-Ig component in the immunoglobulin spectrum.

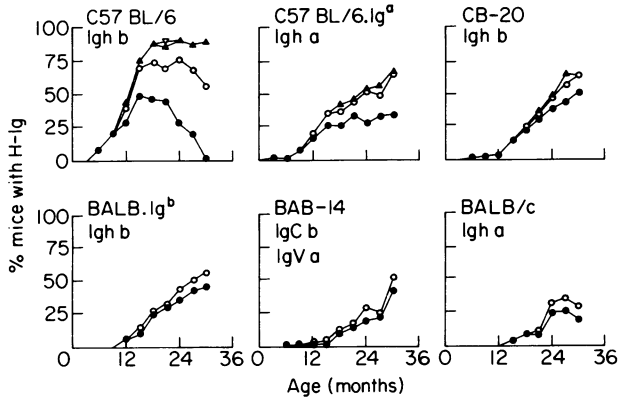

Figure 3. Cumulative representation of single and multiple H-Ig components in the sera of Igh congenic C57BL and BALB mice during ageing. (●), 1 H-Ig; (○), 1–2 H-Ig; (▲), 1–3 H-Ig; (▽), 1–4 H-Ig. IgC, constant part of Igh; IgV, variable part of Igh.

RESULTS

Survival data

The survival data of the six different Igh congenic strains are shown in Fig. 1. All mice had a similar life-span. The 50% survival-time values were 105, 110, 111, 113, 113 and 115 weeks for C57BL/6, BALB.Ig^b, CB-20, C57BL/6.Ig^a, BAB-14 and BALB/c, respectively. No clear association between the survival pattern and the Igh of the congenic mouse strains was found (Fig. 1). During the observation period no serious infections occurred which could have influenced the survival of the mice.

Frequency of H-Ig

The frequencies of H-Ig in the sera of the Igh congenic C57BL and BALB strains increased with ageing, as shown in Fig. 2. C57BL/6 control mice developed H-Ig in the highest frequencies and with an earlier onset than did the other five strains. BALB/c control mice and BAB-14 mice showed the lowest frequencies of H-Ig. The C57BL/6.Ig^a mice showed a lower age-related H-Ig frequency than C57BL/6 control mice, but a somewhat higher one than CB-20 (except at 27 months of age) and BALB.Ig^b mice. The latter two strains developed H-Ig in a higher frequency than BALB/c and BAB-14 mice did. In the sera of C57BL/6 control mice especially, and of some of the other mice, more than one H-Ig component was detected (Fig. 3). The frequency of mice with multiple H-Ig also increased with ageing. Of the Igh-congenic mice, 53% of the C57BL/6, 24% of the C57BL/6.Ig^a, 16% of the CB-20, 10% of the BALB.Ig^b, 8% of the BAB-14 and 6% of the BALB/c mice showed multiple H-Ig in their sera.

Origin of H-Ig

MG form a large, heterogeneous group of B-cell proliferative disorders with a H-Ig component. Most MG can be classified in one of four major categories (Radl, 1985): (i) B-cell malignancies; (ii) BMG; (iii) immunodeficiencies with a T < B immune system dysbalance; and (iv) antigen-driven MG. Only three conditions show a clear-cut relationship with age: Categories (i), (ii) and the immunodeficiency due to ageing group of category (iii). The data presented in Fig. 2 do not allow one to conclude whether the H-Ig observed during ageing belonged to Category (i), (ii) or (iii). Therefore, the MG of all mice with H-Ig were classified on the basis of individual follow-up investigations (Table 2). In this way, the H-Ig present in 89%, 60%, 56%, 48%, 30% and 35% of, respectively, the C57BL/6, C57BL/6.Ig^a, CB-20, BALB.Ig^b, BAB-14 and BALB/c mice were classified according to their origin. Multiple myeloma or Waldenström-like B-cell lymphoma were defined by the serological finding of H-Ig of a high concentration (> 5 g/l), with progression and decreased levels of other immunoglobulins. The morphological patterns of these malignancies were found to be comparable with those in man (Radl *et al.*, 1985a; J. Radl *et al.*, manuscript in preparation). These malignant monoclonal B-cell proliferations [Category (i)] were found or suspected in 2–4% of the mice in all strains. In 6–64% of the mice the H-Ig persisted for longer than 6 months, usually until the death of the animals and without any sign of plasmacellular malignancy. Therefore, these long-lasting H-Ig were considered to fulfill the criteria for BMG [Category (ii)] as known in man (Radl *et al.*, 1978). A single transient monoclonal B-cell proliferation belonging to the immunodeficient Category (iii) was found in 0–8% of the mice. The remaining H-Ig of 14–31% of the congenic mice with H-Ig could not be evaluated in time because the H-Ig appeared in old animals in which death prevented a sufficiently long follow-up.

Since Fig. 2 presents the data of the H-Ig of all categories observed during ageing, it is not possible to draw conclusions from Fig. 2 with regard to the role of the Igh in the development of BMG [Category (ii)]. Using the combined data of Fig. 2 and Table 2 a BMG-frequency figure was constructed (Fig. 4). This figure shows the age-related increase of non-malignant persistent H-Ig, presumably BMG, in the six congenic strains. Taking into account the survival of the mice and the necessary observation period of 6 months for BMG, the construction of Fig. 4 required the omission of the 27- and 30-month time-points. During ageing, the highest frequencies of BMG were found in C57BL/6 mice, the lowest in BALB/c mice. Within the strains showing an intermediate frequency of persistent H-Ig, C57BL/6.Ig^a developed higher, BAB-14 slightly lower frequencies of BMG than CB-20 and BALB.Ig^b mice.

H-Ig isotype distribution

The heavy and light chain isotype distribution of the first appearing persistent H-Ig, presumably BMG [Category (ii); Radl, 1985], is shown in Table 3. The most frequent heavy chain isotypes of the presumed BMG in the C57BL/6 and C57BL/6.Ig^a mice were shown to be the IgG2a and IgG2b isotype, respectively. In the BALB congenic mice the IgM class was revealed to be the most frequent isotype among the first appearing presumed BMG. In contrast, in C57BL/6 and C57BL/6.Ig^a mice the frequencies of IgM were low. In parallel, the BALB Igh-congenic mice, except the BALB/c controls, had low IgG2a serum concentrations (as assessed by immunoelectrophoresis) and a low frequency of persistent H-Ig of the IgG2a isotype. An unexpectedly high frequency of lambda-bearing presumed BMG (25%) was found in CB-20 mice. These mice showed a relatively high incidence (16%) of BMG of the IgM lambda isotype.

Table 2. Distribution (%) of malignant (MM), non-malignant persistent (presumably BMG) and transient H-Ig components detected in the sera of ageing Igh congenic C57BL and BALB mice calculated on the basis of individual follow-up case histories

Mouse strain	Mice (*n*)	H-Ig: MM	Presumably BMG	Transient only	Unclassifiable	Absent	Onset BMG < 12 months*
C57BL/6	53	2	64	6	17	11	53
C57BL/6.Ig^a	83	4	30	7	19	40	44
CB-20	101	2	22	1	31	44	14
BALB.Ig^b	100	2	17	3	26	52	18
BAB-14	86	3	13	0	14	70	18
BALB/c	50	2	6	8	19	65	0

*Frequency (%) of mice with persistent H-Ig starting before 12 months of age.

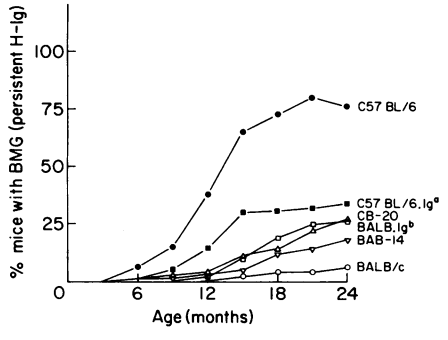

Figure 4. Frequency of non-malignant persistent H-Ig (presumably BMG) in the sera of Igh congenic C57BL and BALB mice during ageing. (●), C57BL/6; (■), C57BL/6.Ig^a; (△), CB-20; (□), BALB.Ig^b; (▽), BAB-14; (○), BALB/c.

A similar pattern of the heavy and light chain isotype distribution was obtained when the H-Ig of all categories [Categories (i)–(iv); Radl, 1985] were included in the calculation.

DISCUSSION

In this study, some influence of loci within the Igh complex on the development of MG in ageing Igh-congenic C57BL and BALB mice was found. None of the five congenic strains approached the H-Ig frequencies of the C57BL/6 control strain. With respect to the onset, occurrence, multiple appearance and persistence of the H-Ig, a decrease from C57BL/6 via C57BL/6.Ig^a, CB-20, BALB.Ig^b and BAB-14 to BALB/c was observed. Regarding the isotype distribution of the presumed BMG, it is remarkable that the major isotype was the same as in the original strain. Thus, it seems that the genetic influence on the development of BMG is very complex and cannot be ascribed simply to a certain H-2 (Van den Akker *et al.*, 1987) or Igh locus. From our data it appeared that the Igh contributes to the development of BMG to some extent; however, probably other subcellular genetic factors, e.g. those which regulate certain clonal cell functions, also contribute to the pathogenesis of BMG. These postulated factors should be related to the C57BL genome. Viral factors intrinsic to the C57BL genome, derepression of genes or gene amplification (Waldenström, 1982, 1983) or the activation and/or expression of particular oncogenes (Goustin *et al.*, 1986; Klein & Klein, 1986), may be among these factors. They all may affect sequences which exert a negative control on cell proliferation, resulting in the monoclonal proliferation of B cells carrying the Igh of C57BL.

The data of the C57BL/6 and BALB/c mice are in agreement with those of C57BL/10.ScSn and BALB/c mice from our previous studies on the influence of H-2 haplotypes on the development of MG (Van den Akker *et al.*, 1987). With respect to the isotype distribution of the presumed BMG, the most frequent in the BALB congenic strains (including BALB/c) was the IgM isotype; this finding is at variance with earlier data (Van den Akker *et al.*, 1987). In the latter study, IgG1 was found to be the most frequent isotype in BALB/c mice. The high IgM lambda frequency among the presumed BMG of CB-20 was another peculiar finding we cannot explain at this moment. Frequencies and isotype distribution of H-Ig in the C57BL/6 mice are fully comparable with those reported earlier for C57BL/KaLwRij mice (Radl *et al.*, 1978).

In the construction of the BMG frequency figure (Fig. 4), BMG was defined as a H-Ig component persisting for longer than 6 months, usually until death and without any sign of plasmacellular malignancy. Evidence that the persistent H-Ig are true BMG should be confirmed by transplantation studies. Persistent H-Ig from C57BL mice could be transplanted up to three or four times into young, healthy syngeneic mice by bone marrow grafting (Radl *et al.*, 1979), indicating an intrinsic defect but a limited life-span of the B-cell clone affected in BMG. So far no data on transplantation of bone marrow cells from BALB/c mice with spontaneously appearing persisting H-Ig are available.

An interesting observation is the difference in MG frequencies between the BAB-14 on the one hand and the BALB.Ig^b and CB-20 mice on the other (Figs 2 and 4). The latter strains showed higher frequencies of H-Ig than BAB-14, while the only genetic difference between these strains concerns the variable part of the Igh (IgV). Since IgV codes for sequences including the antigen-combining site of the immunoglobulin molecule and the idiotype, the way in which mice deal with antigenic stimulation may influence the development of MG. This suggestion is supported by our recent observation, that chronic excessive stimulation with dinitrophenylated human albumin, ovalbumin and pneumococcal polysaccharide resulted in the development of higher frequencies of MG in ageing C57BL mice than in a

Table 3. Heavy and light chain isotype distribution (%) among first-appearing non-malignant persistent H-Ig (presumably BMG) detected in the sera of aging Igh congenic C57BL and BALB mice

Mouse strain	(*n*)	H-Ig observed (*n*)	Presumably BMG (*n*)	%						
				IgM	IgG1	IgG2a	IgG2b	IgG3	IgA	λ
C57BL/6	53	83	34	9	26	33	21	9	2	12
C57BL/6.Ig^a	83	74	25	11	12	11	41	22	3	8
CB-20	101	79	22	40	15	12	22	10	1	25
BALB.Ig^b	100	59	17	31	31	6	19	13	—	12
BAB-14	86	34	9	56	17	10	17	—	—	11
BALB/c	50	24	3	67	—	33	—	—	—	—

non-immunized control group (Van den Akker, Brondijk & Radl, 1988). Although many reports deal with the regulation of the assembly and expression of variable region genes (reviewed by Yancopoulos & Alt, 1986), very little if anything is known about the regulation of the B-cell clone size or immunoglobulin production rate by the IgV.

In conclusion, our data show that factors associated with the allotype of the Igh do not play a dominant role in the development of BMG. Although they may play a contributing role, there must still be other C57BL-related genetic factors regulating proliferative cell functions, which are mainly involved in the development of BMG. Studies on the possible role of oncogene dysfunction in the pathogenesis of BMG using molecular biological and immunological techniques in the C57BL models of BMG and multiple myeloma (Radl *et al.*, 1985b) are in progress.

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