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An Eμ-v-*abl* transgene elicits plasmacytomas in concert with an activated *myc* gene

Helen Rosenbaum, Alan W.Harris, Mary L.Bath, Judy McNeall, Elizabeth Webb, Jerry M.Adams and Suzanne Cory

The Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia

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To clarify how the v-*abl* oncogene of Abelson murine leukemia virus contributes to lymphoid tumorigenesis, we introduced the gene linked to an immunoglobulin heavy chain enhancer (Eμ) into the mouse germline. Although lymphoid development was not detectably affected in young Eμ-v-*abl* mice, three transgenic lines shared a high predisposition to develop clonal plasmacytomas that secreted IgA or IgG. The unexpected absence of pre-B lymphomas suggests that Abelson virus generates such tumors by infecting an early lymphoid progenitor cell that has not yet activated the heavy chain enhancer. Most plasmacytomas bore a rearranged c-*myc* gene, apparently as a result of spontaneous translocation to the *Igh* locus. Moreover, progeny of a cross with analogous Eμ-*myc* mice rapidly developed oligoclonal plasmacytomas. Thus, the collusion of v-*abl* with c-*myc* is stage specific, efficiently transforming plasma cells but not pre-B cells or B cells.

Key words: v-*abl*/c-*myc*/oncogene synergy/plasmacytomagenesis/transgenic mice

Introduction

Several forms of the *abl* tyrosine kinase have been associated with hematologic malignancies (reviewed by Konopka and Witte, 1985; De Klein *et al.*, 1988). The rapidly lymphomagenic Abelson murine leukemia virus expresses a *gag–abl* fusion protein which lacks the N-terminal portion of the normal cellular *abl* gene product, as does *bcr–abl*, the hybrid gene generated by the 9;22 chromosome translocation characteristic of human chronic myeloid leukemia and some cases of acute lymphoblastic leukemia. Both the *gag–abl* and *bcr–abl* proteins have heightened tyrosine kinase activity.

The lymphomagenic effects of Abelson virus are complex (Whitlock and Witte, 1985). Neonatal mice are generally susceptible, but adults of most inbred strains are resistant (see Risser, 1982). Whereas pre-B cell lymphomas arise rapidly after i.p. or i.v. injection of virus, intrathymic injection produces T cell lymphomas (Cook, 1982). Moreover, infection of BALB/c mice treated with the mineral oil pristane accelerates the onset of plasmacytomas carrying a chromosome translocation that couples the c-*myc* gene to an immunoglobulin locus (Potter *et al.*, 1973; Ohno *et al.*, 1984).

Expression of v-*abl* does not directly transform normal lymphocytes to full malignancy. Bone marrow or fetal liver cells infected *in vitro* with Abelson virus require prolonged culture before tumorigenic pre-B cell lines emerge (Whitlock *et al.*, 1983a) and infected animals exhibit polyclonal cell growth from which clonal pre-B lymphomas evolve (Green *et al.*, 1987a). It therefore seems likely that malignancy requires alterations in cellular genes that complement v-*abl* activity. Since the strain of helper virus can profoundly affect lymphomagenesis by the replication defective Abelson virus (Rosenberg and Baltimore, 1978; Savard *et al.*, 1987), insertion of the helper virus may sometimes alter a critical cellular gene. The proviral insertion site *Ahi*-1 recently identified in some Abelson pre-B lymphomas (Poirier *et al.*, 1988) may harbor such a gene.

To study the lymphoid transforming potential of v-*abl* in the absence of complicating variables such as the helper virus and the accessibility of target cells, we have introduced the gene into the mouse germline under the control of the immunoglobulin heavy chain enhancer (Eμ). The efficacy of this regulatory element in promoting transgene expression in B and T lymphoid cells has been demonstrated in a variety of transgenic mouse lines (e.g. Grosschedl *et al.*, 1984; Adams *et al.*, 1985; Rosenbaum *et al.*, 1989; Van Lohuizen *et al.*, 1989). Surprisingly, the v-*abl* transgene has no observable effect on lymphoid development, and the mice display a remarkable predisposition to develop tumors of the plasma cell, the end stage of B cell differentiation. Whereas the basis for the emergence of malignant clones in most transgenic models remains an enigma (Cory and Adams, 1988), we find that the tumors of Eμ-v-*abl* mice display a common genetic alteration: a rearranged c-*myc* gene. Unexpectedly, the oncogenic cooperativity of v-*abl* and *myc* appears to be stage specific, because v-*abl* mice provided with a *myc* transgene rapidly develop plasmacytomas but no tumors earlier in the B lineage.

Results

Eμ-v-abl transgenic mice develop plasmacytomas

To target lymphoid cells for v-*abl* expression, the *gag–abl* fusion gene was introduced into a vector harboring the immunoglobulin heavy chain enhancer and an SV40 promoter, small intron and polyadenylation sequence (Figure 1). Microinjection of the *Eco*RI fragment containing the expression cassette into fertilized (C57BL/6 × SJL) F1 eggs produced seven primary transgenic animals. Two died suddenly, before they had been mated, and could not be autopsied informatively. Another unbred primary male (v-*abl* 40) became ill with a plasmacytoma, but a transgenic line was established from his sperm by *in vitro* fertilization. The four remaining primary transgenic animals founded lines, but only those generated from v-*abl* 8 and v-*abl* 1 produced tumors.

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Eμ-v-*abl* mice display a remarkable predisposition to develop plasmacytomas; all autopsies on sick mice from three generations of the v-*abl* 40 and v-*abl* 8 lines revealed tumors of this type. The v-*abl* 1 primary mouse proved to harbor more than one active transgene. Remarkably, one subline (designated v-*abl* 2) develops only plasmacytomas and the other (v-*abl* 1) only lymphomas. The lymphomagenic line will be described elsewhere. Thus, of four active Eμ-v-*abl* lines, three develop plasmacytomas exclusively.

Some of the gross abnormalities revealed by autopsy are illustrated in Figure 2A–C, and the findings from 58 ill animals are summarized in Table I. Although the presentation was variable and differed somewhat between the three lines, most of the sick mice contained one to three swollen lymph nodes, usually including the mesenteric and pancreatic nodes, which could be enlarged up to 50-fold. Tumor nodules often appeared on the peritoneal connective tissue. The sole overt abnormality in three animals was a tumor mass under the roof of the skull. Some 10% of the mice developed hind limb paralysis without any overt pathology, but histologic examination revealed spinal cord compression by tumor masses in the vertebral canal (Figure 2D).

Histologic analysis of 44 animals with advanced disease established that the abnormal tissues were replete with medium to large, mitotically active cells. These cells were often readily identifiable as large plasma cells (Figure 2E) by their rounded shape, eccentric nucleus, abundant strongly stained cytoplasm and juxtanuclear clear zone, which sometimes contained eosinophilic material. The nuclear chromatin distribution frequently resembled the 'cartwheel' or 'clockface' characteristic of normal plasma cells. Some tumors (Figure 2F) exhibited a less distinctively plasmacytic cell type; although these cells were also medium to large (8–12 μm diameter) with open-appearing nuclei and well stained cytoplasm, they were cohesive and lacked the Golgi zone and characteristic chromatin pattern. Mitotic activity within the 44 tumors ranged from 0.5 to 18 mitoses per average high power field (3–120 mitoses per mm^2).

Apart from the tumor masses, only the bone marrow and the spleen were prominent sites of plasmacytoma invasion. Sections of sternum and vertebral column revealed partial to complete replacement of normal marrow elements by tumor cells in 82% of the animals. Non-invaded regions of marrow typically contained a 2- to 4-fold higher proportion of granulocytes than normal (Figure 2G). The red pulp of the spleen was usually invaded by tumor cells and also contained large numbers of normoblasts and granulocytes,

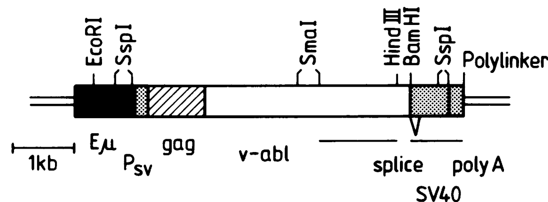

Fig. 1. Structure of the Eμ-v-*abl* transgene. The *Eco*RI fragment used for oocyte injection contained the immunoglobulin heavy chain enhancer (Eμ), the SV40 early region promoter (P_{SV}), the *gag–abl* gene and the splice, termination and polyadenylation sequence for SV40 early transcripts (see Materials and methods). Lines below the construct indicate the location of the v-*abl* probe, a *Sma*I–*Hin*dIII fragment, and the SV40 probe, a subcloned *Bam*HI fragment of the transgene.

a sign that extramedullary hematopoiesis was compensating for impaired bone marrow function. Some animals (8/44) displayed a classic byproduct of plasmacytoma, swollen kidney tubules filled with eosinophilic casts (Figure 2H), while the splenic white pulp in five of these cases and in three others was surrounded by amorphous eosinophilic material (Figure 2I) identified as amyloid by its affinity for Congo Red.

As a test of malignancy, $1-2 \times 10^6$ plasmacytoma cells were transferred into histocompatible recipients by s.c. and i.p. injection. Five of seven v-*abl* 40 and five of eight v-*abl* 8 tumors initiated progressive tumor growth, the latent period varying from 20 to 95 days. Seven of these tumors were simultaneously injected into recipients pretreated with pristane, since pristane-induced plasmacytomas can be efficiently transplanted only in pristane-treated recipients (Potter *et al.*, 1984), but the pristane priming did not shorten the latent period or increase the frequency of Eμ-v-*abl* tumor 'takes'. Tumors in the recipients grew in peritoneal connective tissue and within the spleen. The mesenteric, lumbar and pancreatic lymph nodes were often massively enlarged by tumor cells, and the bone marrow was occasionally invaded. Subcutaneous tumors sometimes developed at the site of injection.

The plasmacytomas are clonal and secrete IgA or IgG

In confirmation of the diagnosis of plasmacytoma, serum from transgenic animals with advanced tumors contained abundant discrete immunoglobulins (see below). Of the 43 sera analysed by electrophoresis, 34 yielded a single abnormal band, while seven samples yielded two such bands. Thus, the tumors appeared to be monoclonal or biclonal. Isotype analysis on the sera indicated that 15 produced IgA, eight IgG_{2b}, three IgG_1 and two IgG_{2a}. None produced IgM, IgD or IgE. Two were non-producers, and four were untypable with the reagents employed. The remaining nine tumors produced elevated levels of both an IgA and an IgG.

Further confirmation of the B lymphoid origin and clonality of the tumors was provided by analysis of their immunoglobulin and T cell receptor gene loci (data not shown). Whereas only one tumor had a rearranged T cell receptor γ locus, all 25 analysed displayed rearrangement within the *Igh* locus and 17 analysed at the κ locus also showed rearrangement. Most tumors displayed two rearranged J_H-bearing fragments and either one or two new C_κ-bearing fragments, evidence that each tumor arose from a single transformed cell.

Transgene expression in tumors and preneoplastic tissues

All of the tumors tested expressed the ~5 kb polyadenylated transcript expected from the transgene, as well as low levels of a somewhat smaller RNA (Figure 3A). Transgene expression in both the v-*abl* 40 and v-*abl* 8 tumors was comparable to that of v-*abl* in Abelson virus-induced pre-B (ABLS-8) and plasmacytoma (WEHI-267 and ABPC17) lines and ~15- to 30-fold higher than that of the endogenous c-*abl* transcripts in non-Abelson-infected lines such as the T lymphoma ST4 (Figure 3A).

As expected, the transgene was also expressed in the hematopoietic tissues of non-tumorous Eμ-v-*abl* mice (Figure 3B). The presence of Eμ-v-*abl* RNA in the bone marrow, spleen, mesenteric lymph node and thymus suggests that the

transgene is active in both the B and T cell lineages. Indeed, especially in the v-*abl* 40 mice, expression seemed highest in the thymus. Within the spleen and lymph node, Eμ-v-*abl* expression was similar to that of the endogenous c-*abl* gene in the v-*abl* 40 line and 3- to 5-fold higher than this in the v-*abl* 8 line.

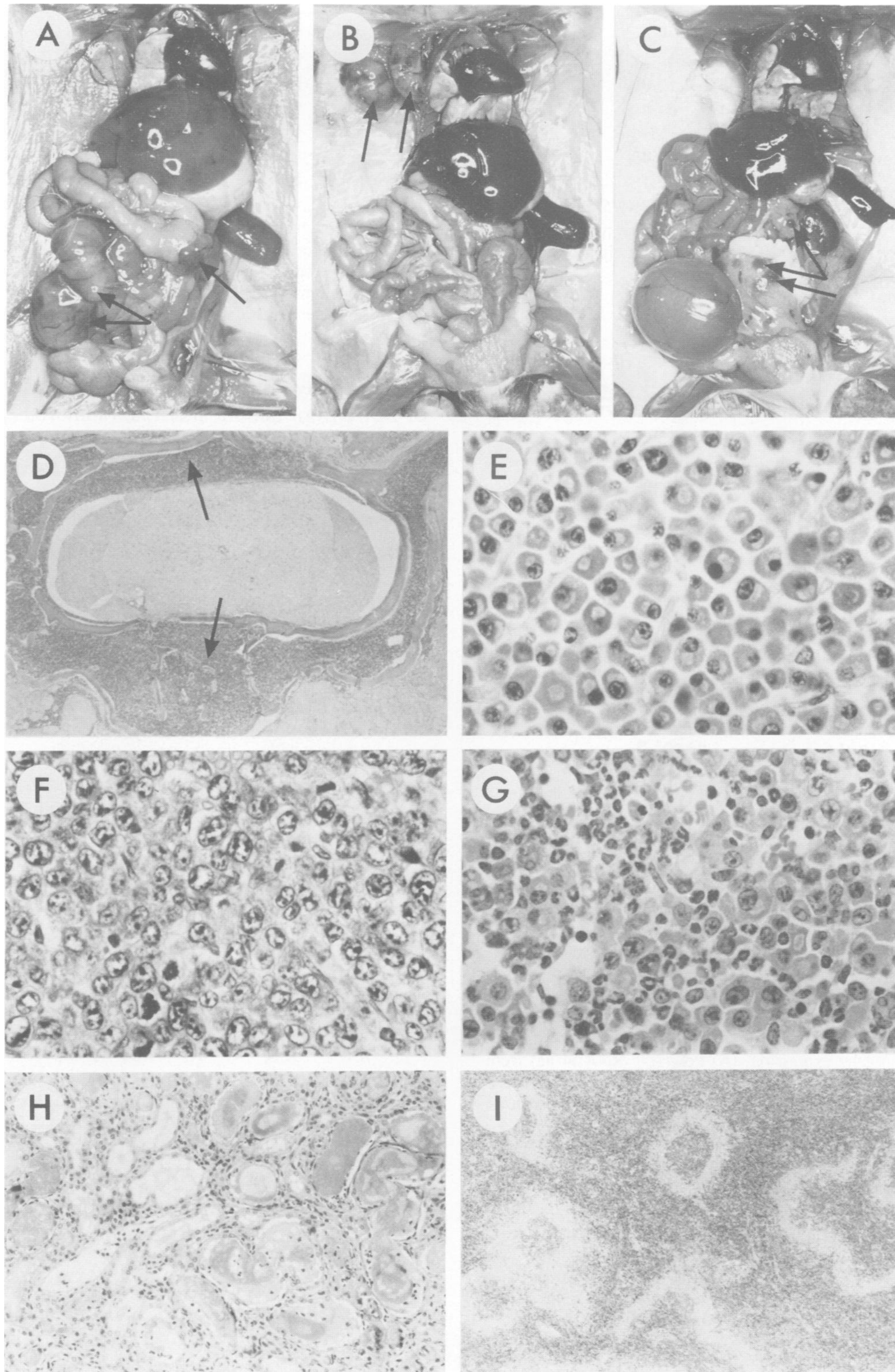

Fig. 2. Pathology of Eμ-v-*abl* disease. Dissection of mice killed when ill reveals **A**, massive enlargement of the mesenteric lymph node and Peyer's patch, or **B**, enlargement of two peripheral lymph nodes (the right brachial and axillary nodes), or **C**, an enlarged bladder and small tumor foci in peritoneal fat, but no gross abnormality of lymphoid organs, accompanying hind limb paralysis. Histology reveals **D**, plasmacytoma growth in the bone marrow and epidural space of the lumbar vertebral column of a v-*abl* 40 mouse with paralysed hind limbs (×25); **E**, plasmacytoma cells of 'classic' morphology in enlarged mesenteric lymph node (×500); **F**, plasmacytoid immunoblastic lymphoma with high-frequency mitosis (×500); **G**, plasmacytoma cells and granulocytes in sternal bone marrow (×500); and examples of **H**, 'myleoma kidney' (×50); and **I**, amyloid deposits around splenic white pulp zones (×25).

Table I. Gross pathology of Eμ-v-*abl* mice with advanced tumors

Feature	Incidence		
	v-*abl* 40	v-*abl* 8	v-*abl* 2
Enlarged mesenteric lymph node	85%	37%	87%
Abdominal tumor mass	35%	46%	75%
Enlarged spleen	42%	33%	62%
Enlarged peripheral lymph node	23%	4%	12%
Peritoneal ascites	27%	4%	12%
Hind limb paralysis	23%	37%	12%
Thoracic vertebral tumor	19%	4%	0%
Subparietal tumor	8%	21%	0%
Tumor transplantability	71% (5/7)	63% (5/8)	ND

Results from autopsy of 25 v-*abl* 40, 24 v-*abl* 8 and eight v-*abl* 2 mice.
ND = not determined.

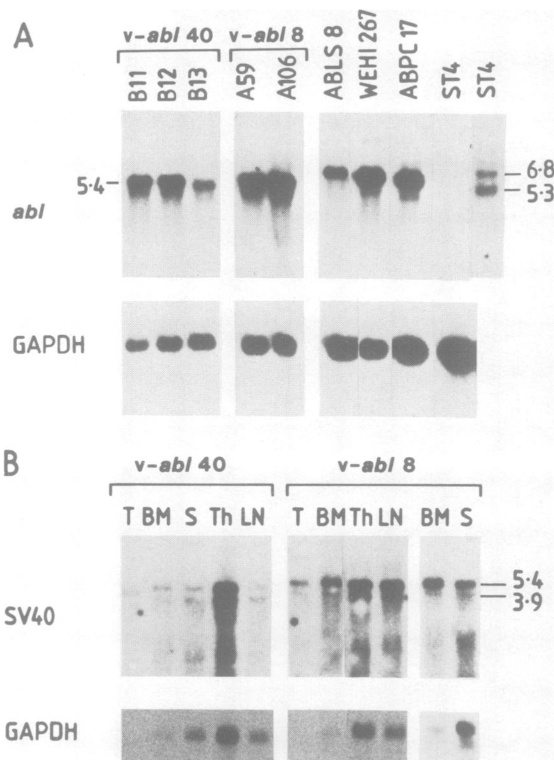

Fig. 3. Eμ-v-*abl* transgene expression. **(A)** Poly(A)$^+$ RNA (4 μg) from the indicated cell lines and Eμ-v-*abl* tumors was analysed by Northern blot hybridization, using the *abl* probe (see Figure 1) and then with a probe for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). ABLS-8 is an Abelson virus induced pre-B lymphoma cell line, while WEHI-267 and ABPC 17 are Abelson induced plasmacytomas. Non-Abelson lymphoid lines, here illustrated by T lymphoma cell line ST4, express c-*abl* at a level barely detectable with the exposure used for the v-*abl*-expressing line; a 10-fold longer exposure of this track is shown on the right. **(B)** Poly(A)$^+$ RNA from hematopoietic tissues of five pre-tumorous Eμ-v-*abl* mice or Eμ-v-*abl* plasmacytomas was hybridized to the SV40 probe (Figure 1) and then to a GAPDH probe. The signal obtained from 4 μg of RNA from spleen (S), thymus (Th) and mesenteric lymph node (LN) and all RNA obtained from the bone marrow (BM) is compared with that from 0.1 μg of RNA from v-*abl* 40 or v-*abl* 8 tumors (T). Sizes are given in kilobases.

Tumors contained substantially higher amounts of transgene mRNA than did the preleukemic tissues, as 20- to 40-fold less RNA gave comparable autoradiographic signals (Figure 3B). Hence, elevated transgene expression may be

Table II. Cellular composition of lymphoid tissues from pretumorous v-*abl* 40 mice and normal mice

Tissue		Cell surface marker[a]			
		Ig	B220	Thy-1	Mac-1
Bone marrow	Normal	10 ± 3	32 ± 11	<2	38 ± 10
	v-*abl* 40	10 ± 3	32 ± 14	<2	34 ± 8
Spleen	Normal	49 ± 7	44 ± 10	30 ± 9	ND
	v-*abl* 40	52 ± 8	43 ± 7	27 ± 6	ND
Lymph node	Normal	22 ± 5	19 ± 6	75 ± 6	ND
	v-*abl* 40	21 ± 6	20 ± 7	80 ± 8	ND
Thymus	Normal	ND	<2	99 ± 1	ND
	v-*abl* 40	ND	<2	100 ± 0	ND

[a]The mean percentage ± SD of cells bearing the indicated marker. The v-*abl* 40 values derive from four mice, and the normal values from two age-matched (C57BL/6 × SJL)F1 animals.
ND = not determined. Similar results were obtained with v-*abl* 8 mice.

a prerequisite for tumor onset or progression. Southern blots of DNA from tumors and a non-tumorous tissue (kidney) with v-*abl* and SV40 probes, however, provided no evidence of transgene rearrangement, and only a few tumors displayed a slightly elevated copy number (~2-fold). Comparison with the endogenous c-*abl* gene suggested that all three lines of Eμ-v-*abl* mice bear ~5 transgene copies per cell.

Lymphocyte development is unperturbed prior to tumor onset

To assess whether lymphoid development was altered before tumors arose, tissues from young v-*abl* 40 and v-*abl* 8 mice free of overt disease and lacking transplantable tumor cells were analysed by immunofluorescence and flow cytometry. Significantly, no abnormalities were detected in the cellular composition of their bone marrow, spleen, lymph nodes or thymus (Table II). The cell size distributions were normal (not shown), as were the proportions of pre-B cells (B220$^+$, Ig$^-$), B cells (B220$^+$, Ig$^+$), T cells (Thy-1$^+$) and cells of the granulocyte–macrophage lineage (Mac-1$^+$).

c-myc is rearranged in Eμ-v-abl plasmacytoma

Since oil-induced plasmacytomas of BALB/c mice almost invariably harbor a c-*myc* translocation (Ohno *et al.*, 1984; Cory, 1986), we tested whether the context of c-*myc* was altered in the Eμ-v-*abl* plasmacytomas. Southern blots with a *myc* probe revealed that most tumor DNAs did yield an altered *Eco*RI fragment (Figure 4A). Sixteen of the 18 v-*abl* 40 plasmacytomas tested had a *myc* rearrangement detectable in either *Eco*RI or *Eco*RV digests, as did eight of 10 v-*abl* 8 and three of four v-*abl* 2 tumors.

The region of the *Igh* locus that has recombined with c-*myc* in ~80% of BALB/c plasmacytomas bearing a 12;15 translocation is the Cα gene (Cory, 1986). Significantly, 12 of 25 Eμ-v-*abl* plasmacytomas tested contained a rearranged Cα-bearing *Eco*RI fragment indistinguishable in size from the rearranged *myc* fragment (Figure 4B). Thus, many apparently harbor a *myc* gene coupled to the Cα locus (Figure 4D), presumably as a result of a 12;15 chromosome translocation. The other *myc* rearrangements may reflect recombination at other sites within the *Igh* locus or with a light chain locus.

Most 12;15 translocations in oil-induced plasmacytomas disrupt the 5′ portion of the c-*myc* gene, evicting its promoters to the reciprocal recombinant chromosome. That

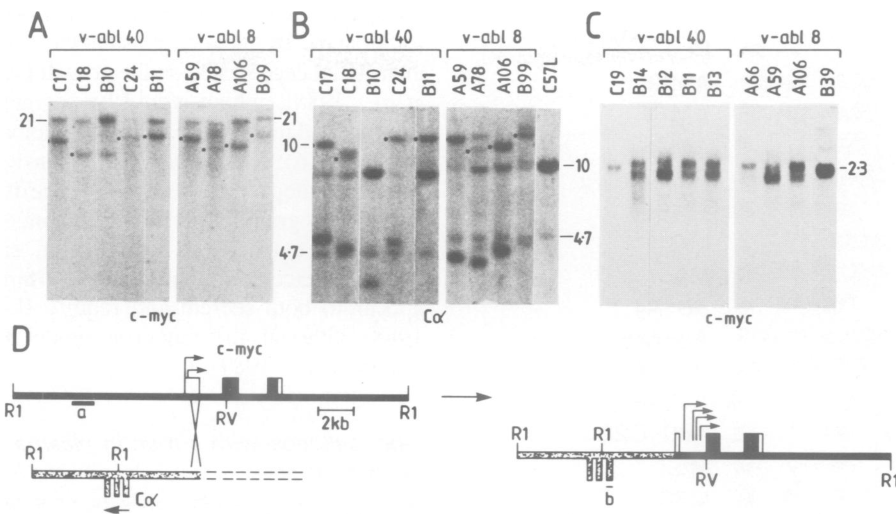

Fig. 4. Most Eμ-v-*abl* plasmacytomas contain a rearranged c-*myc* gene. (**A**) Southern blot analysis of *Eco*RI digested DNA (15 μg) from plasmacytomas arising in the indicated Eμ-v-*abl* mice, using a c-*myc* cDNA probe. The v-*abl* 40 and v-*abl* 8 DNAs were run on separate gels. (**B**) Rehybridization of the stripped filters used in A with a 5′ Cα cDNA probe corresponding to the genomic exon 1 (b in Figure 4D). This probe hybridizes to the expected 10 kb *Eco*RI fragment in (unrearranged) C57BL/6 liver (C57L) DNA, but is contaminated with some 3′ Cα sequences so it also detects (weakly) the 3′ 4.7 kb Cα fragment. Dots mark rearranged Cα-bearing fragments indistinguishable in size from rearranged c-*myc*-bearing fragments. (**C**) Northern blots of poly(A)$^+$ RNA (4 μg) from the indicated Eμ-v-*abl* plasmacytomas, using the c-*myc* cDNA probe. The mobility of the normal ~2.3 kb c-*myc* mRNA is indicated. (**D**) Diagrammatic representation of recombination between the c-*myc* and immunoglobulin Cα loci. Only one product is shown, that linking Cα and the c-*myc* coding exons, which are in opposite transcriptional orientation, as indicated by arrows. Probe *a* is a *Hin*dIII fragment from the 5′-flanking region of c-*myc*.

region is also disrupted in the Eμ-v-*abl* tumors, since *Eco*RI plus *Eco*RV digests contained novel 5′ *myc* fragments (detected with probe *a* of Figure 4D). Analysis of RNA confirmed this conclusion (Figure 4C). Plasmacytomas apparently lacking a *myc* rearrangement (e.g. v-*abl* 8 B39, v-*abl* 40 C19) displayed the normal ~2.3 kb *myc* transcripts, while those harboring a rearrangement displayed transcripts of varied sizes, presumably reflecting initiation at multiple sites within *myc* intron 1 or the incoming DNA, as in oil-induced BALB/c plasmacytomas.

An Eμ-myc transgene accelerates plasmacytomagenesis

Plasmacytoma development in Eμ-v-*abl* mice required several months. Figure 5 shows that 60% of the v-*abl* 40 mice became ill between 11 and 52 weeks of age (when monitoring was terminated). The incidence in v-*abl* 8 mice initially appeared lower but subsequent breeding segregated an inactive and an active transgene; in the active subline, the incidence within the first 8 months (30%) has been comparable to that in v-*abl* 40 mice.

To test whether translocation of *myc* to the *Igh* locus is the rate-limiting step in plasmacytoma development, we crossed Eμ-v-*abl* and Eμ-*myc* mice. All the doubly transgenic progeny of such a cross with v-*abl* 40 became ill with plasmacytomas between 5 and 8 weeks of age (median 45 days) (Figure 5). An analogous v-*abl* 8 cross gave a comparable acceleration (median 59 days). Significantly, none of 25 such animals developed pre-B or B lymphomas, the tumors that arise spontaneously in Eμ-*myc* mice, even though Eμ-*myc* mice bear a greatly expanded pool of cycling pre-B cells (Adams *et al.*, 1985; Langdon *et al.*, 1986). Thus, *myc*/v-*abl* oncogenic synergy appears to be stage specific (see Discussion).

The plasmacytomas of the doubly transgenic mice were strikingly prominent in the small intestine. Bowel obstruc-

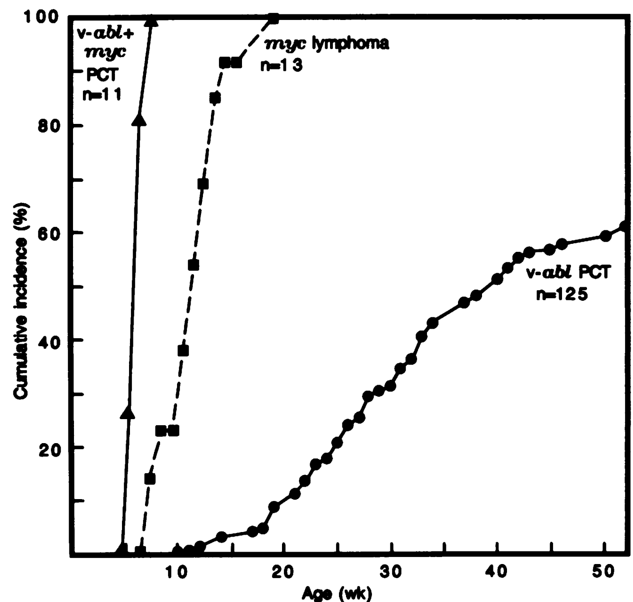

Fig. 5. Kinetics of tumor onset in v-*abl* 40 mice and in v-*abl* 40-Eμ-*myc* mice. The plots were generated from 125 v-*abl* 40 mice of both sexes, which were killed and autopsied upon becoming ill, and from 11 v-*abl* 40-Eμ-*myc* animals and 13 of their Eμ-*myc* littermates.

tion, due to telescoping contractions over plasmacytoma masses (intussusception), was the cause of sudden terminal illness in most animals (22/25), and histology revealed foci of plasmacytoma cells at numerous other sites in the bowel wall of each mouse. In 64%, the mesenteric lymph node was also enlarged by engorgement with similar cells, but there were few or none in the bone marrow. The altered presentation (cf. Table I) suggests that most of these tumors originate from gut-associated plasma cells.

Unlike the tumors carrying spontaneous *myc* rearrangements in Eμ-v-*abl* mice, those in Eμ-v-*abl*/*myc* mice

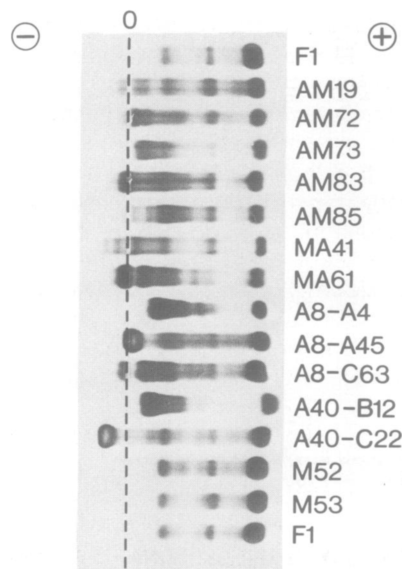

Fig. 6. Electrophoretic analysis of serum proteins from Eμ-v-*abl* and from Eμ-v-*abl*/*myc* mice with advanced tumors. F1 denotes serum from non-transgenic (C57BL/6 × SJL)F1 mice; AM and MA samples were from the transgenic cross, M52 and M53 inherited only the Eμ-*myc* transgene and developed lymphomas; A8- and A40-samples were from mice carrying only the Eμ-v-*abl* transgene.

appeared to be oligoclonal and often to secrete IgM. Their serum usually contained several abnormal immunoglobulins (Figure 6) and of 17 tested by class-specific assay against normal serum, an elevated level of IgG, IgA and IgM was found in nine, of both IgG and IgA in six and of both IgG and IgM in one. Southern blots provided additional evidence that more than one clone was present. The κ locus was particularly informative. Whereas a single rearranged band was seen with most Eμ-v-*abl* tumors, two or three bands were typical of tumors from the doubly transgenic animals (data not shown).

No IL-6 mRNA detected in Eμ-v-abl tumors

For human plasma cell myeloma, production of small amounts of the hematopoietic growth factor interleukin-6 (IL-6) has been implicated as an autocrine stimulus of tumor growth (Kawano *et al.*, 1988). We therefore tested Eμ-v-*abl* plasmacytomas for expression of the IL-6 gene. While the 1.3 kb IL-6 mRNA was readily detectable by Northern blot analysis of RNA from P388D1 cells, a common experimental source of IL-6, none was apparent in nine Eμ-v-*abl* tumors under conditions which would have revealed ~1/30 of the P388D1 level (data not shown).

Discussion

Spontaneous plasmacytomas are extremely rare in the mouse but arise with high incidence in certain inbred strains (BALB/c and NZB) after oils such as pristane have been introduced into the peritoneal cavity (reviewed by Potter *et al.*, 1984). The Eμ-v-*abl* lines of (C57BL/6 × SJL) mice described here provide a valuable new animal model for plasmacytomagenesis in which the strain restriction has been breached. The incidence and rate of tumor onset is similar to that in pristane-treated BALB/c mice, 60% of the animals showing plasmacytoma at 11–52 weeks of age (Figure 5). Unlike pristane-induced tumors, the Eμ-v-*abl* tumors transplant readily in untreated normal mice. The requirement for pristane by induced plasmacytomas probably involves their initial dependence on the growth factor IL-6 (Van Snick *et al.*, 1988), which would be secreted by macrophages accumulated in the oil-reactive granuloma tissue (Nordan and Potter, 1986). If, as seems likely, Eμ-v-*abl* plasmacytomas are IL-6 independent because they arose in the absence of an induced granuloma, their autonomy does not appear to result from autocrine IL-6 production, since no IL-6 mRNA could be detected in the tumors. Certain human myelomas appear to both secrete and require IL-6 (Kawano *et al.*, 1988), although this autocrine model has been challenged (Klein *et al.*, 1989).

v-abl colludes with c-myc in plasma cells but not pre-B or B cells

It is clear that Eμ-v-*abl* transgene expression was insufficient to generate plasmacytomas. Young mice expressed the transgene (Figure 3B) but contained no tumor cells. Indeed they showed no detectable disturbance to their lymphoid cell populations (Table II). Moreover, most tumors were clonal and their onset was sporadic. The striking observation that at least 80% of them contain a rearranged c-*myc* gene (Figure 4) provided strong evidence that v-*abl* and *myc* cooperate in the transformation of plasma cells and suggested that the rate-limiting step might be the chance occurrence of a *myc* translocation within a single cell amongst the susceptible population. This notion was confirmed when mice bearing both an Eμ-v-*abl* and an Eμ-*myc* transgene displayed greatly accelerated plasmacytoma onset (Figure 5).

Our data complement previous findings (Potter *et al.*, 1973; Ohno *et al.*, 1984) that the development of plasmacytomas carrying *myc* translocations in pristane-treated BALB/c mice is accelerated by infection with Abelson virus. The strain restriction on pristane induction is not understood, but might reflect a higher probability of *myc*/Ig locus translocation in BALB/c (and NZB) mice. On this hypothesis, crossing the Eμ-v-*abl* transgene onto a BALB/c background should accelerate plasmacytomagenesis.

As in the oil-induced plasmacytomas, the most common class of immunoglobulin expressed by the Eμ-v-*abl* tumors was IgA (55% of tumors), although tumors expressing IgG were nearly as frequent. The absence (or rarity) of IgM-expressers is also a common feature of the two systems (Potter *et al.*, 1984) and may reflect a critical role for the switch recombination machinery in translocating the *myc* gene (Gerondakis *et al.*, 1984). Consistent with this possibility, plasmacytomas which are not dependent on an endogenous gene translocation, such as those arising in Eμ-v-*abl*/*myc* mice or those induced by a *myc* retrovirus (Potter *et al.*, 1987; Clynes *et al.*, 1988), commonly secrete IgM.

Other oncogene combinations can also favor plasmacytoma development. Plasmacytomas arose rapidly in pristane-treated mice infected with a retrovirus bearing Eμ-*myc* and v-H-*ras* (Clynes *et al.*, 1988) or one bearing the v-*myc* and v-*raf* genes (Potter *et al.*, 1987; Troppmair *et al.*, 1989). Hence, plasmacytomagenesis appears to be promoted by *myc* acting in concert with any of three different types of cytoplasmic oncogene product: the v-*abl* tyrosine kinase, the v-*ras* GTPase or the v-*raf* serine/threonine kinase.

How these oncogene combinations promote plasmacytomagenesis is not clear. Our studies on Eμ-*myc* transgenic

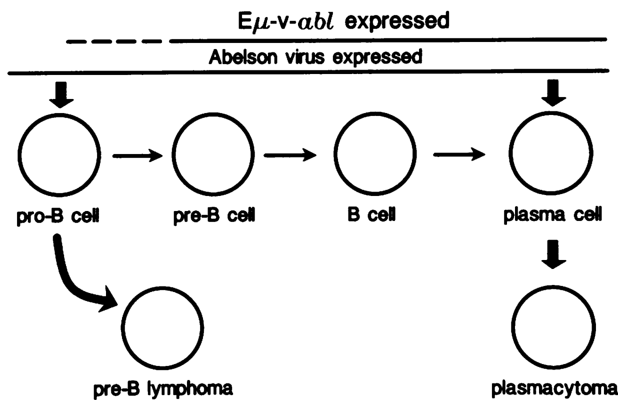

Fig. 7. Model for the preferential generation of plasmacytomas in Eμ-v-*abl* transgenic mice, It is postulated that v-*abl* oncogenicity is largely confined to two stages in the B lineage, the plasma cell and a primitive lymphoid progenitor, which might correspond to the pro-B cell (the immediate precursor of the pre-B cell) or to a more primitive lymphoid stem cell. The Abelson virus can infect both the plasma cell and the early lymphoid targets, but an Eμ-driven transgene will not usually be active at the earlier stage.

mice suggest that constitutive *myc* expression encourages lymphocytes to proliferate but does not relieve their need for exogenous growth factors (Langdon *et al.*, 1988). Hence, a major function of a cooperating cytoplasmic oncogene product may be to free a *myc*-driven plasma cell from factor dependence. It is well established that v-*abl* can relieve hematopoietic cells of a requirement for IL-3 (e.g. Cook *et al.*, 1985; Pierce *et al.*, 1985), and that v-*mil* (avian *raf*) frees avian myeloid cells of dependence on their cognate growth factor (Graf *et al.*, 1986). Moreover, v-H-*ras* and v-*raf* can each render early Eμ-*myc* B lineage cells autonomous (Alexander *et al.*, 1989). Whether autonomy is achieved by autocrine growth factor production is unclear. With v-*mil*, the autocrine model is strongly supported (Graf *et al.*, 1986) and, while autocrine models have not been favored by most studies with v-*abl*, Hariharan *et al.* (1988) recently showed that v-*abl* and *bcr–abl* induce production of small amounts of IL-3.

Are v-*abl* and an activated *myc* gene sufficient to elicit a fully malignant plasma cell directly? That question, which has not been fully resolved for any oncogene combination in any cell type, is particularly difficult to address for lymphoid cells because of their extensive migration throughout the body. Certainly the v-*abl*/*myc* combination in the doubly transgenic mice rapidly elicited multifocal, oligoclonal tumors. On the other hand, some plasma cells must have had a marked growth advantage, because the resulting tumors arose focally and did not appear to represent unrestrained growth of every plasma cell. An attractive possibility is that proliferation of particular clones is favored by antigenic stimulation. If so, *myc* and *abl* activation may be the only necessary genetic alterations, although an additional mutation during clonal expansion cannot be ruled out. Curiously, the Eμ-v-*abl*/*myc* animals invariably displayed tumor masses in the gut wall, whereas nearly all the spontaneous tumors of v-*abl* mice arose elsewhere in the body (Table I). This finding may mean that the doubly transgenic tumors arise *in situ* shortly after contact of B cells with cognate environmental antigens.

The human equivalent of murine plasmacytoma is plasma cell myeloma (multiple myeloma) but little is known about its molecular basis. *Myc* translocations are rare (Sümegi *et al.*, 1985; Gould *et al.*, 1988), but some 30% of tumors contain a mutated N-*ras* oncogene (see Seremetis *et al.*, 1989). The predilection of Eμ-v-*abl* for plasmacytomagenesis shown in the present work suggests that *abl* should be evaluated for a role in the human disease.

Significantly, the collusion of v-*abl* and c-*myc* appears to be stage specific, as we have found no evidence that these genes can collaborate in transforming B lineage cells at the pre-B or B cell stage. Most compellingly, no lymphomas arose in Eμ-v-*abl*/*myc* doubly transgenic animals. Moreover, targets for Abelson virus transformation are not elevated in the bone marrow of the Eμ-*myc* transgenic mouse (Dyall-Smith and Cory, 1988; Alexander *et al.*, 1989), despite its several fold higher pre-B cell content (Langdon *et al.*, 1986). Similarly, infection of newborn Eμ-*myc* mice with Abelson virus did not accelerate lymphoma formation (although it did induce a plasmacytoma), whereas viruses bearing v-H-*ras* or v-*raf* very rapidly generated pre-B lymphomas (Langdon *et al.*, 1989). The apparent impotence of the *abl*/*myc* combination in pre-B and B cells may be due to a lack of oncogenic activity of v-*abl* at these differentiation stages (see below). Collaboration between *myc* and v-*abl* has not been clearly demonstrated in other cell types, but certain Abelson virus-transformed fibroblast lines contain an amplified c-*myc* locus (Nepveu *et al.*, 1985), and v-*myc* augments the oncogenic impact of *bcr–abl* on Rat-1 (but not NIH3T3) fibroblasts (Lugo and Witte, 1989).

Why do Eμ-v-abl and Abelson virus preferentially transform different cells?

Given that the Abelson virus is most noted for its ability to generate pre-B and T lymphomas, it is surprising that no tumors of these types have appeared in the three Eμ-v-*abl* lines described here. This transgene is clearly capable of generating such tumors, because they do arise in the v-*abl* 1 line, as will be described elsewhere. Because pre-B lymphomas are generated by an almost identical vector bearing the N-*myc* gene (Rosenbaum *et al.*, 1989), it seems unlikely that the lack of lymphomas in most Eμ-v-*abl* lines reflects a failure of these mice to express the transgene in pre-B cells. Moreover, Eμ-v-*abl* transcripts are readily detectable in the bone marrow, spleen, lymph node and thymus of pre-tumorous animals (Figure 3B). It might be argued that the generation of lymphomas by Abelson virus requires insertional activation of cooperating cellular genes. While that notion would be consistent with the helper virus effects on Abelson lymphomagenesis (see Introduction) and the preferred Moloney provirus insertion site in some Abelson lymphomas (Poirier *et al.*, 1988), it does not jibe well with the ability of helper virus-free Abelson virus to generate pre-B lymphomas (Green *et al.*, 1987b; Dyall-Smith and Cory, 1988).

The model that best accounts for our data is that v-*abl* transformation of early B (or T) lineage cells requires its initial expression within a progenitor cell in which the IgH enhancer (and hence the Eμ-v-*abl* transgene) is not yet active (Figure 7). (Presumably the transgene in the exceptional v-*abl* 1 line is activated abnormally early by some influence of flanking DNA.) Recent cell fractionation studies also suggest that the major target of transformation by Abelson virus is a very primitive cell (Tidmarsh *et al.*, 1989). In our model, the typical Abelson pre-B lymphoma arises

not by direct transformation of a pre-B cell but by differentiation of the transformed progenitor cell. The primary target may be lineage committed, or a bipotential cell able to generate a T or pre-B lymphoma depending on its microenvironment. The transformation by Abelson virus of certain pre-B and B cell clones from long-term bone marrow cultures (Whitlock *et al.*, 1983b) might indicate that v-*abl* also has some activity at those stages, but those clones were almost certainly partially transformed prior to infection (Whitlock and Witte, 1985). *In vitro* transformation of normal B lymphocytes by Abelson virus requires prolonged culture with mitogenic lipopolysaccharide and is highly inefficient (Serunian and Rosenberg, 1986). The absence of B lymphomas in any strain of Eμ-v-*abl* mice argues that the B cell stage is not susceptible *in vivo*. Thus, the oncogenic action of v-*abl* may be largely restricted to two stages of lymphoid development: the plasma cell and a primitive lymphoid progenitor (Figure 7). The unexpected stage specificity of v-*abl* action may mean that the v-*abl* polypeptide interacts with a gene product present only at particular maturation stages.

Materials and methods

Construction of Eμ-v-abl mice

The lymphoid expression vector (pEμSV2 in Figure 1) used here is a predecessor to that in Rosenbaum *et al.* (1989). Upstream from the SV40 early region promoter (P_{SV}) (a 200 bp *Sph*I−*Hin*dIII fragment from residues 128 to 5171) lies the 985 bp *Xba*I fragment spanning the murine *Igh* enhancer (residues 1−987; Gillies *et al.*, 1983), in opposite orientation to that found in the *Igh* locus. Downstream is the SV40 region containing the small T intron (*Sau*IIIA fragment 4700−4100) and the termination and polyadenylation sequence for SV40 early transcripts (*Sau*IIIA−*Bam*HI fragment 2771−2533). Between the SV40 promoter and splice sequence, inserted as a blunted fragment, is the 3.94 kb *Pvu*I−*Bam*HI fragment containing both *gag* and v-*abl* sequences (residues 865−4805, Reddy *et al.*, 1983), obtained from the Abelson (p120) provirus λAM-1 (Srinivasan *et al.*, 1981). As the construct was assembled in pUC18, some polylinker sites remain at the 3′ end.

Prior to injection, the vector was digested with *Eco*RI endonuclease (see Figure 1) and the Eμ-v-*abl* expression cassette was separated from plasmid sequences by electrophoresis in low melting point agarose. The fragment was then purified (Rosenbaum *et al.*, 1989) and microinjected (at 6 μg/ml) into the fertilized eggs of (C57BL/6JWehi × SJL/JWehi) F1 hybrid (BSF1) mice as described by Brinster *et al.* (1985). Primary transgenic mice and offspring bearing the transgene were identified by dot hybridization to DNA from the tails of weanling mice (Brinster *et al.*, 1985) using a ^{32}P-labeled SV40 probe (a subcloned *Bam*HI fragment spanning the terminal SV40 sequence in the transgene; see Figure 1). Lines were maintained by mating transgenic animals with normal BSF1 mice.

Production of Eμ-v-abl/myc transgenic mice

Mice from the v-*abl* 8 or v-*abl* 40 lines were mated with animals of a C57BL/6JWehi-backcrossed (N10) subline of the Eμ-*myc* 292-1 transgenic line. Doubly transgenic progeny were identified by the hybridization of their DNA to both the SV40 probe (see above) and a pUC12 plasmid probe, which detects sequences present in the Eμ-*myc* transgene (Adams *et al.*, 1985).

Characterization of hematopoietic tissues, tumors and immunoglobulin

Flow cytometric analysis of immunofluorescence and cell size was performed as described by Langdon *et al.* (1986). The absence of malignant cells from the bone marrow, spleen and lymph nodes of young putatively pre-tumorous transgenic animals was verified by the failure of $5-10 \times 10^6$ cells to initiate tumor growth in normal BSF1 mice within a 4 month observation period. DNA and RNA preparation and analysis were as in Rosenbaum *et al.* (1989). Serum proteins were fractionated electrophoretically in Multitrac agarose films (Ciba Corning, Palo Alto, CA) and stained with Coomassie. Initial screens for major immunoglobulin heavy chain isotypes used a Misotest kit (Commonwealth Serum Laboratories, Parkville, Victoria, Australia). Further analysis was by a monoclonal antibody-based ELISA (PharMingen, San Diego, CA) on serum diluted 1/10^4. Biclonal and initially untypable immunoglobulins were assessed further by immunofixation tests in agarose films (Ciba Corning) with antisera specific for mouse IgA, IgG, IgM, IgD and IgE (Nordic, Tilburg, The Netherlands).

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