The 5T mouse multiple myeloma model: absence of c-myc oncogene rearrangement in early transplant generations

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Summary Consistent chromosomal translocations involving the c-myc cellular oncogene and one of the three immunoglobin loci are typical for human Burkitt's lymphoma, induced mouse plasmacytoma (MPC) and spontaneously arising rat immunocytoma (RIC). Another plasma cell malignancy, multiple myeloma (MM), arising spontaneously in the ageing C57BL/KaLwRij mice, was investigated in order to see whether the MM cells contain c-myc abnormalities of the MPC or RIC type. Rearrangement of the c-myc oncogene was found in the bone marrow cells only in 5T2 MM transplantation line in a mouse of the 24th generation and in none of the seven other MM of the 5T series which were of earlier generations. Since the mouse 5T MM resembles the human MM very closely, including the absence of consistent structural c-myc oncogene abnormalities, it can serve as a useful experimental model for studies on the aetiopathogenesis of this disease.

Consistent chromosomal translocations involving the c-myc cellular oncogene and one of the three immunoglobulin loci have been reported in human Burkitt's lymphoma, in induced mouse plasmacytoma (MPC) and in spontaneously arising rat immunocytoma (RIC) (reviewed by Croce & Nowell, 1985; Potter, 1986; Pear et al., 1986; Enrietto, 1987). Translocation is believed to play an important role in the development of these tumours (Klein, 1986). Multiple myeloma (MM) in humans is a neoplasm of B cells at a differentiation stage comparable to that of MPC and RIC. However, a rearrangement of the c-myc oncogene was reported only in three cases of MM (Gazdar et al., 1986; Selvanayagam et al., 1988) and in one case of plasma cell leukaemia (Yamada et al., 1983).

In recent years, several transplantation lines derived from spontaneously arising MM in ageing C57BL mice became available in our institute. This mouse MM resembles the human disease very closely in several aspects (Radl et al., 1988). It is of interest to investigate whether the cells of the experimental 5T MM series contain c-myc abnormalities of the MPC type or whether they resemble the human MM in this respect. The results of this study show that rearrangement of the c-myc oncogene was found in the bone marrow of only one of the 5T MM transplantation lines and was possibly due to a late event in the progression of this malignancy.

Materials and methods

Mice

Male and female C57BL/KaLwRij mice from the colony of the Institute for Experimental Gerontology in Rijswijk, The Netherlands, were used in this study. Detailed information on this inbred strain of mice has been published elsewhere (Zurcher et al., 1982; Van Zwieten et al., 1981).

5T mouse multiple myeloma

The different 5T MM originated spontaneously in ageing C57BL/KaLwRij mice (Radl et al., 1988). The individual 5T MM were further propagated by intravenous transfer of bone marrow or spleen cells into young recipients of the same strain. An attempt was also made to grow the individual 5T MM in an ascitic form by transplanting bone marrow cells

into the peritoneal cavity of young recipient mice. In four instances this was successful. The main characteristics of the individual 5T MM lines pertinent to this study are given in Table I.

Cell preparations

Cell suspensions from bone marrow and spleen were prepared as described (Croese et al., 1987). The percentage of 5T MM cells in these samples was estimated by morphology, cytoplasmic immunoperoxidase staining and by analysis of the cellular DNA content. Cytoplasmic examination was performed on cytocentrifuge preparations (Hijmans et al., 1965) of the suspensions, using PO-labelled antibodies (Nordic Immunological Laboratories, Tilburg, The Netherlands) specific for the isotype of the given 5T MM immunoglobulin (Table I). The DNA from bone marrow or spleen cells was stained with propidium iodine (PI) according to Taylor (1980). The cellular DNA content was analysed by measuring the PI fluorescence intensity by a fluorescence-activated cell sorter (FACS-II, Becton Dickinson, Mountain View, CA, USA).

Southern blot analysis

High molecular weight DNA was extracted from $0.5-1.0 \times 10^8$ bone marrow, spleen or ascitic cells by the method of Kunkel et al. (1977). The chromosomal DNA was digested with the restriction enzymes HindIII or EcoRI under conditions recommended by the manufacturer (Gibco-BRL, Breda, The Netherlands). Digested DNA was electrophoresed on 0.6–0.8% agarose gels in buffer consisting of 89 mM Tris, 89 mM boric acid and 0.2 mM EDTA, pH 8.0 and transferred to Gene Screen Plus filters in 0.4 N NaOH and 0.6 M NaCl (Reed & Mann, 1985). Filters were prehybridised for 2 h at 65°C in a solution of 50 mM Tris/HCl, pH 7.5, 10 mM EDTA, 1 M NaCl, 1% SDS, 0.1% sodium pyrophosphate, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 100 µg ml^{-1} salmon sperm DNA and hybridised in the same solution overnight with 25 ng of c-myc probe, with specific activity of approximately 10^9 c.p.m. µg^{-1} DNA, a 1.4 kb ClaI-EcoRI fragment containing the third exon of the human c-myc gene, which was random-primed ^{32}P-labelled (Boehringer, Mannheim, FRG). After hybridisation, filters were washed to a stringency of 0.5 × SSC (1 SSC = 75 mM NaCl, 7.5 mM sodium citrate, 1% SDS) 0.1% sodium pyrophosphate at 65°C, and exposed overnight at −70°C to Kodak XAR-5 X-ray films. A schema of the murine c-myc gene and the relevant restriction sites are shown in Figure 1.

Table I C57BL/KaLwRij mouse 5T multiple myeloma lines of spontaneous origin

5T MM no.	Isotype	Transplantation generation	Growth pattern	Remark
5T2	IgG2a-k	24 (17)	moderately progressive	several sublines (also ascitic form)
5T7	IgG2b-k	7	'smouldering MM'	
5T13	IgG2b-k	4	moderate	
5T14	IgG1-k	10 (59)	aggressive	different sublines (also ascitic form)
5T21	IgD-k	11	atypical	
5T30	IgG2a-k	3 (3)	aggressive	(also ascitic form)
5T33	IgG2b-k	5 (34)	moderately progressive	(also ascitic form)
5T41	IgG3-k	1	moderate	

Generations of the MM in ascitic form are given in parentheses.
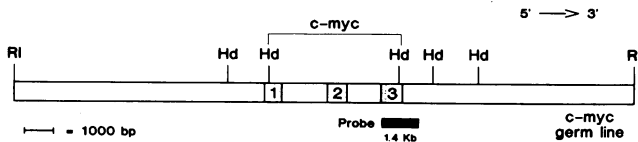
Figure 1 Molecular map of the murine c-myc gene. Restriction sites: RI, EcoRI; Hd, HindIII. The third exon probe used is denoted by a black box.

Figure 2 Autoradiogram of DNA from bone marrow cells digested with HindIII (lanes 1–7) and EcoRI (lanes 8–14). Lanes 1 and 8, 5T2; lanes 2 and 9, 5T7; lanes 3 and 10, 5T14; lanes 4 and 11, 5T33; lanes 5 and 12, 5T41; lanes 6 and 13, RPC-20; lanes 7 and 14, normal bone marrow cells. Analyses of MM 5T13, 5T21 and 5T30 are not shown. For details see the text.

Sensitivity of the technique and controls

Bone marrow cells from normal mice and from RPC-20 mouse plasmacytoma with a known c-myc rearrangement were used as controls. The detection sensitivity limit of the c-myc rearrangement in this technique was determined by admixing isolated SP2/0 hybridoma cells to normal bone marrow cells in different proportions and was found to be about 3–4%. In the different 5T MM preparations, the percentages of MM cells varied from 15 to 70% and from 7 to 44% for bone marow and spleen cells, respectively.
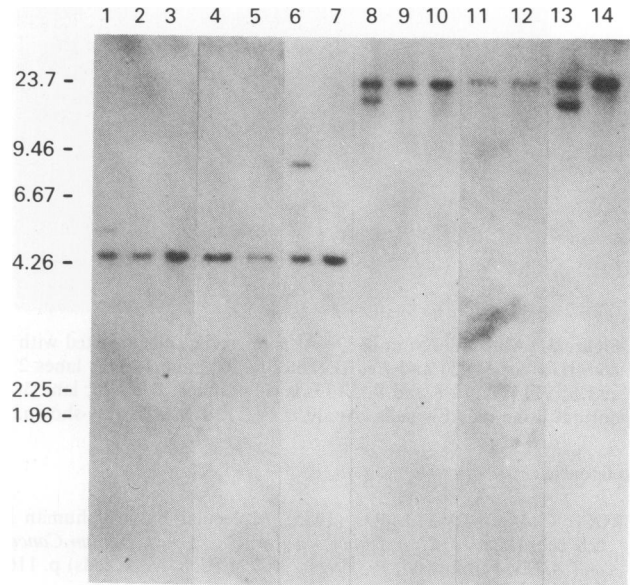
Results

No rearrangement of the c-myc oncogene was found in any of the individual 5T MM (Table I) when spleen cells were investigated. Similar results were obtained when bone marrow cells were analysed, however, with one exception. The mouse 5T7, 5T13, 5T14, 5T21, 5T30, 5T33 and 5T41 MM showed only the germ line fragments of 4.6 kb (HindIII) and 22 kb (EcoRI) as in normal mouse bone marrow (Figure 2). However, the 5T2 MM showed an additional hybridising fragment of 5.4 kb (HindIII) and 15 kb (EcoRI). The same pattern was observed when DNA from 5T2 MM cells originating from an animal with an ascitic form of 5T2 MM was investigated (Figure 3). This indicates that the c-myc rearrangement took place in the common donor of both sublines in an earlier generation.

Three other 5T MM were shown to be able to grow in the peritoneal cavity of unprimed recipient mice: 5T14, 5T30 and 5T33 MM. The DNA isolated from the 5T30 and 5T33 MM showed only the germ line configuration, while that of the 5T14 MM produced an additional band of 1.3 kb (HindIII) and 5.8 kb (EcoRI) (Figure 3).

Discussion

The most common structural alteration in human Burkitt's lymphoma, mouse plasmacytoma and rat immunocytoma is an interruption of the c-myc gene upstream of its second exon (Mushinski et al., 1987). In many MPC, the breakpoint is within the first intron between E1 and E2, in some MPC, the breakpoint in c-myc occurs 300–500 base pairs 5′ of E1 (Potter, 1986). This kind of abnormality, indicated by a rearrangement of the c-myc oncogene within the bone marrow tumour cells of the 5T MM series was found only in the

5T2 MM bearing mouse in the 24th transplantation generation and in none of the seven other 5T MM, which were of earlier generations. The bone marrow is the major site of this malignancy in both man and the C57BL mouse (Radl et al., 1988). Therefore, any structural abnormalities within different oncogenes, if they were of basic importance for the development of this malignancy, should primarily be present in the MM cells of the bone marrow compartment. In humans, rearrangement of the c-myc oncogene was found only in three cases (one of them being a very progressive IgA MM involving pleural tissue) and in one case of plasma cell leukaemia (Gazdar et al., 1986; Selvanayagam et al., 1988; Yamada et al., 1983). In this context, it is interesting that in the 5T14 MM, being able to grow in the peritoneal tissue, a rearrangement of the c-myc was found in ascitic cells but not in the bone marrow cells. Moreover, the 5T2 MM in an advanced stage can develop features of a plasma cell leukaemia (Ebbeling et al., 1985). These findings indicate that structural abnormalities of the c-myc oncogene of the most common MPC types are not a prerequisite for the development of MM. Our data, together with those of others on human MM, can be interpreted as indicating that such rearrangement can take place, possibly as a late event in the progression of this malignancy or due to its location in peritoneal or pleural tissue, where it can obtain selective growth advantage. Our investigation does not exclude some other structural abnormalities which would occur outside the analysed region.

Cytogenetic investigations performed in this multiple
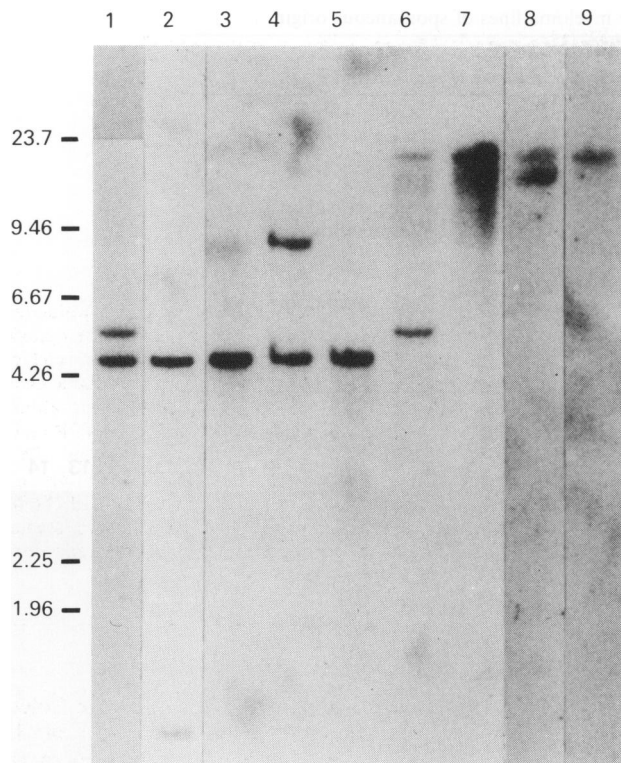
Figure 3 Autoradiogram of DNA from ascitic cells digested with HindIII (lanes 1-5) and EcoRI (lanes 6-9). Lane 1, 5T2; lanes 2 and 6, 5T14; lanes 3 and 9, 5T33; lanes 4 and 8, RPC-20; lane 5, normal bone marrow cells. Analysis of 5T30 MM is not shown.

myeloma of the 5T series (Th.W. van den Akker et al., in preparation) showed near triploid chromosome numbers in four lines (5T2, 5T7, 5T14 and 5T41) and hypotetraploid numbers in one (5T33). All karyotypes showed one or two copies of normal chromosome 15 and markers involving chromosome 15. 5T2 and 5T14 (transplant generation 11) showed markers with partial deletion of chromosome 15. No consistent abnormalities involving chromosomes 6, 12 or 16 with the three immunoglobulin gene loci were found. To detect more subtle changes, if present, within the first exon of the myc gene, studies on the myc RNA message will be performed after establishing cell lines of the 5T multiple myelomas in vitro (work in progress).

The human MM and the mouse 5T MM show a close resemblance in several aspects (Radl et al., 1985, 1988), including possibly also the c-myc pattern. Therefore, these 5T MM series offer an excellent experimental model for studies on the aetiology and pathogenesis of multiple myeloma. In addition, this mouse B cell malignancy, expressed mainly at the differentiation stage of a plasma cell, shows clear-cut differences when compared with MPC and RIC, both also involving a B cell at its last differentiation stage (Radl et al., 1988). Investigation of these differences may shed new light on the possible microheterogeneity of the plasma cell and its malignant counterparts evolving either into local plasmacytoma or diffuse multiple myeloma.

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