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Depletion of the predominant B-cell population in immunoglobulin μ heavy-chain transgenic mice

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The transgenic mouse line M54 was generated by introducing a functionally-rearranged immunoglobulin μ heavy-chain gene into the germ line of a C57B1/6 inbred mouse[1]. Previous examination of the antibodies produced by B-cell hybridomas derived from transgenic M54 mice showed that the presence of the μ transgene grossly altered the immunoglobulin repertoire of unimmunized animals, suggesting that these mice suffer from a serious immunoregulatory perturbation[2]. Studies presented here introduce a new perspective on this functional defect. We show that the lymphoid tissues from these transgenic mice lack virtually all conventional bone-marrow-derived B cells, which constitute the predominant B-cell population in normal mice and which typically produce primary and secondary antibody responses to T-cell-dependent antigens[3–6]. Moreover, the bone marrow from transgenic M54 mice is depleted of pre-B lymphocytes, indicating a serious defect in early B-cell lymphopoiesis. In contrast, CD5 (Ly-1) B cells, a second B-cell population displaying a characteristic set of cell surface markers which are derived from distinct precursors in the peritoneum[3–7], are represented at normal frequencies in these transgenic mice. Thus, the presence of the rearranged immunoglobulin heavy-chain transgene in M54 mice results in an unexpected selective developmental defect that impairs the development of bone-marrow-derived pre-B and B cells without affecting Ly-1 B cells.

M54 transgenic mice produce and secrete substantial amounts of endogenously encoded (Igh-6b allotype) antibodies of all isotypes[1,2]. They also produce clearly detectable levels of transgene-encoded (Igh-6a allotype) immunoglobulin M (IgM) heavy chains containing a NP^a idiotype variable region which is specific for the hapten 4-hydroxy-3-nitrophenyl (NP)[1,2,8,9]. Two striking observations have been made concerning immunoglobulin gene expression in these animals[2,9]. First, a large proportion of the antibodies produced by hybridomas derived from M54 mice express endogenous heavy-chain variable region (V_H) genes that are rarely expressed in normal C57B1/6 mice[9] and display idiotypes which cross-react with the NP^a idiotype encoded by the transgene. Secondly, rearrangement of endogenous immunoglobulin heavy-chain genes is partially blocked; unrearranged endogenous Igh alleles exist in 40% of pre-B-cell clones derived from M54 bone marrow by transformation with Abelson murine leukaemia virus (A-MuLV)[2].

These findings led us to inquire whether individual B-cell populations are affected to different extents by the presence of the μ transgene. Two such B lymphocyte subsets have been distinguished by their characteristic cell surface markers and functional properties[3–6]. Conventional B cells have intermediate to high levels of surface IgM and IgD, high levels of B220/6B2 (Ly-5) but lack the CD5 (Ly-1) and MAC-1 antigens. Cells of this surface phenotype are repopulated from progenitors in the bone marrow and produce most of the commonly studied anti-

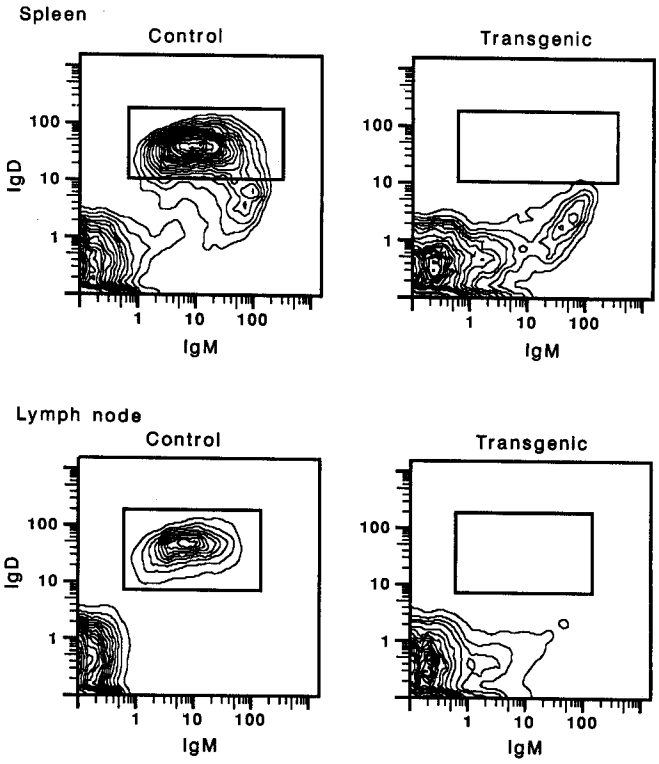

Fig. 1 Conventional B cells are depleted in the spleen and lymph nodes of IgM heavy-chain transgenic (M54) mice. Cells from spleen and peripheral lymph nodes of six-month-old transgenic mice and normal littermates were examined by fluorescence activated cell sorter (FACS) analysis. The box in each panel delineates the conventional (bright IgD) B cell population. Spleen and lymph node cells were prepared as previously described and stained with monoclonal antibodies: anti-IgM, 331.12/FITC; and anti-IgD (Igh-5b), AF6-122/biotin; or anti-B220, RA3-6B2/biotin; or anti-MAC-1; MAC-1/biotin [14]. Biotin-conjugated antibodies were revealed with Texas Red/avidin. Purification and fluorochrome conjugation of the monoclonal antibodies has been described[15]. The anti-IgM (331.12) recognizes both the Igh-6a and Igh-6b alleles of IgM[16]. Dead cells were excluded by propidium iodide staining[17]. FACS analyses were conducted as previously described[7]. Data are presented as 5% probability contour maps[18].

body responses in normal mice[3,4]. Ly-1 B cells, in contrast, have high levels of IgM, low levels of IgD, and express the B220/6B2, MAC-1 and CD5 (Ly-1) surface antigens (A.M.S., unpublished data)[3]. These cells appear to be self-maintaining in the peripheral immune system in that they are repopulated from immunoglobulin-bearing progenitors present in the peritoneum and thus have been suggested to comprise a separate B-cell lineage[4–6]. CD5 (Ly-1) B cells produce the majority of autoantibodies[3,5,7] and a large proportion of the serum immunoglobulin in normal animals[3]. Furthermore, they apparently tend to use a restricted set of V_H genes that includes the V_H genes expressed in the hybridomas derived from the transgenic mice[3,10].

To determine the proportions of individual B-cell types within lymphatic tissues of M54 transgenic mice, we examined cell-surface marker expression using fluorescent antibodies and flow cytometry with the fluorescence activated cell sorter (FACS). Table 1 and Fig. 1 show that the B cells bearing high levels of surface IgM and IgD (IgM^+IgD^{bright}) are virtually absent from the spleen and lymph nodes of M54 mice. In normal mice, these cells comprise the major fraction of conventional B cells[3,5]. The few B cells detectable in the transgenic spleen and lymph nodes express intermediate to high levels of surface IgM but have little or no surface IgD (see Fig. 1). The lineage relationship of these

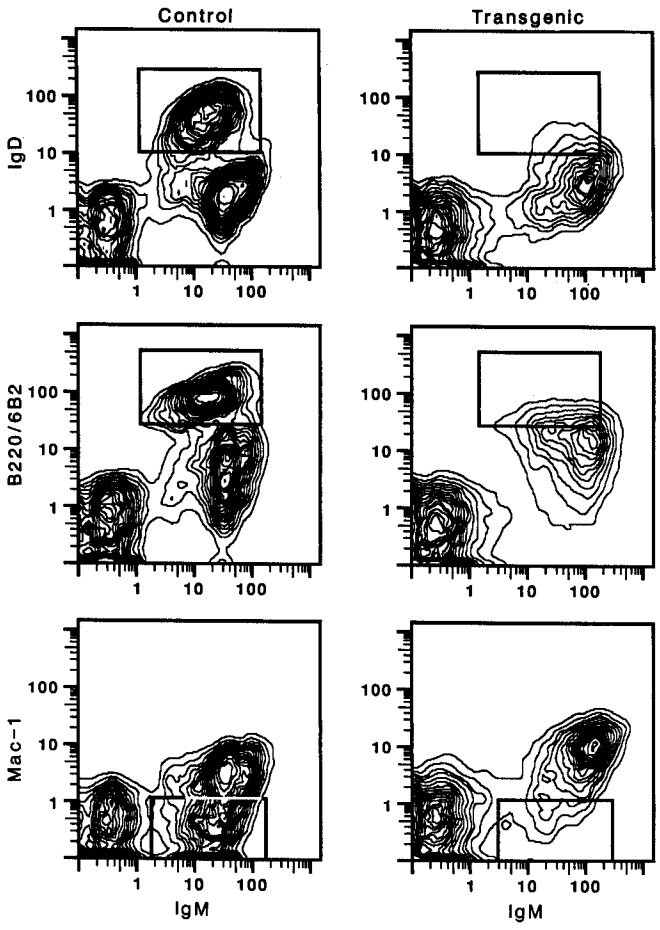

Fig. 2 Conventional B cells are selectively depleted in the peritoneum of IgM heavy-chain transgenic (M54) mice. The cell-surface phenotype of peritoneal B cells from six-month-old transgenic mice and normal littermates was characterized by FACS analysis as described in Fig. 1. The box in each panel delineates the conventional B-cell population.

cells, which express a surface phenotype rarely seen in normal mice, remains to be established.

To quantify CD5 (Ly-1) B-cell representation, we analysed peritoneal cell suspensions from control C57B1/6 and transgenic M54 mice because CD5 (Ly-1) B cells are present at substantially higher frequencies in the peritoneum than in the spleen[3,4]. Table 1 and Fig. 2 show that cells bearing high levels of surface IgM and little or no surface IgD (IgM^+IgD^{dull}) are represented at normal frequencies in the peritoneum of transgenic mice, whereas cells of the IgM^+ IgD^{bright} surface phenotype, characteristic of conventional B cells, are virtually missing. To demonstrate that the IgM^+IgD^{dull} cells are not conventional B cells exhibiting an IgD^{dull} surface phenotype due to an allelic exclusion of the endogenous μ/δ heavy-chain locus by the μ transgene, we used two other criteria for the characterization of these cells. First, we found that the levels of expression of the B220/6B2 and MAC-1 cell surface markers are characteristic of CD5 (Ly-1) B cells (Fig. 2). This combination of cell surface antigens has proven more diagnostic for members of the CD5 (Ly-1) B-cell population than the presence or absence of the CD5 (Ly-1) antigen itself because a fraction of CD5 (Ly-1) B cells do not express the CD5 (Ly-1) antigen[3]. In the transgenic mice studied here, the CD5 (Ly-1) antigen was expressed on the surface of 50% of the peritoneal IgM^+IgD^{dull} cells (data not shown). Second, FACS studies with anti-IgM allotype reagents that distinguish between transgene-encoded (Igh-6a allotype) and endogenously encoded (Igh-6b allotype) IgM demonstrated that 80% of peritoneal B cells of M54 mice co-express both types of μ heavy chains (A.S. *et al.*, manuscript in preparation). Thus, the presence of the μ transgene in M54 mice leads to a selective deficiency in the number of conventional B cells without affecting CD5 (Ly-1) B cells. These findings were confirmed by analysis of two additional M54 mice, and similar results were obtained with several mice from the independently derived M95 and M52 mouse lines which carry the same μ transgene (data not shown)[1].

T-cell populations in the transgenic animals were characterized in spleen, lymph nodes and peritoneum by determining the proportions of cells bearing CD4 and CD8 (L3T4 and Ly-2) surface markers. Both T-cell subsets were represented at approximately normal frequencies (Table 1). The cells appeared

Table 1 Numbers of total and individual lymphocyte populations in M54 transgenic and C57B1/6 control mice

	Total lymphocytes recovered	IgM^+ B cells		T cells	
		Bright IgD	Dull IgD	$CD4^+$	$Lyt\text{-}2^+$
Peritoneum					
Control	12	4.0	4.8	0.6	0.6
Transgenic	8	0.2	4.3	0.8	0.7
Spleen					
Control	147	63.2	21.0	27.0	15.0
Transgenic	45	0.5	9.0	12.0	12.0
Lymph node					
Control	8	2.8	0.2	2.3	2.0
Transgenic	16	0.2	1.6	6.1	7.0

Numbers of lymphocytes ($\times 10^6$) were obtained from integrations of FACS analyses and represent the average of two mice. Cells were stained with monoclonal antibodies: anti-IgM, 331.12/FITC and anti-IgD, AF6-122/biotin or anti-CD8 (Lyt-2) 53-7.8/FITC and anti-CD4 (L3T4), GK-1.5/biotin. Staining of cells and FACS analyses were carried out as described in Fig. 1.

phenotypically identical to T cells in control mice by FACS analysis (data not shown). Thus, the lymphopoiesis defect in M54 transgenic mice does not affect the lymphoid T-cell lineage.

We also examined the B cells in the bone marrow of M54 mice, to determine the proportions of the B cell subsets. Conventional bone marrow pre-B cells are detectable either as $B220^+$, IgM^- cells[11] or as cells that generate relatively small forward and wide-angle light scatter signals (A.M.S., unpublished observation). The number of typical pre-B cells in transgenic bone marrow was reduced sixfold (Fig. 3). Thus, the presence of the transgene seriously diminished the steady-state level of the immediate precursor cell type for most or all normal conventional B cells.

The mechanism responsible for the selective interference with bone marrow lymphopoiesis in these mice is unclear. The depletion of conventional B cells in M54 mice could be due to a rapid turnover of these cells or their progenitors, or to a direct block in B-cell development. The underlying mechanisms for either kind of defect could be intercellular, via interaction with T lymphocytes, or independent of cell-cell interactions. The marked deficiency of pre-B cells in the bone marrow suggests that the transgene exerts its influence through an intracellular mechanism perhaps related to the blockade of endogenous gene rearrangements in conventional B-cell precursors previously described[2]. If so, CD5 (Ly-1) B cells may be less sensitive to the presence of the μ transgene, perhaps because the strategy for gene rearrangement and allelic exclusion used by these cells is fundamentally different from that used by conventional B cells. This hypothetical explanation is consistent with experimental evidence demonstrating V_H segment replacement into rearranged *VDJ* gene units in differentiating CD5 (Ly-1) B tumour cells[12].

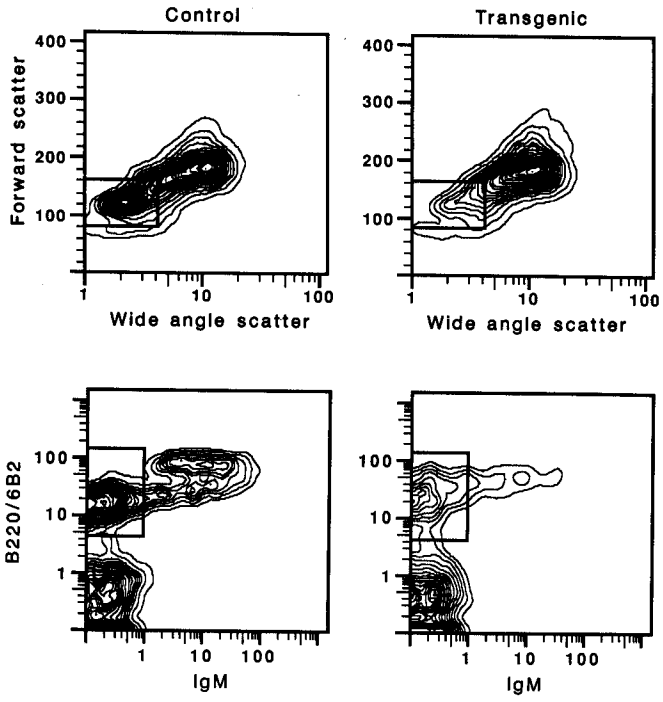

Fig. 3 Pre-B cells are depleted in the bone marrow of IgM heavy-chain transgenic (M54) mice. Bone-marrow cells from six-month-old mice were stained as in Figs 1 and 2. The top panels present the forward angle versus wide angle light scatter of total bone-marrow cells. Pre-B cells have the low forward and wide angle light scatter shown in the boxes in the top panels. The bottom panels present the IgM versus B220/6B2 profiles of the cells within the scatter gates (boxes) shown in the top panels. The boxes in the lower panels delineate the IgM^-, $B220^+$ pre-B cells which constitute 12% of the 6.7×10^7 control and 2.5% of the 5.1×10^7 transgenic bone-marrow cells.

The selective lack of conventional B cells in the transgenic mice potentially explains several anomalies in the previous data. For example, we noted that the transgenic mice gave rise to pre-B lymphoid A-MuLV transformants that all express the transgene and have partially inhibited rearrangement of their endogenous immunoglobulin heavy-chain genes[2]. By contrast, the B-cell hybridomas derived from transgenic mice often do not express the transgene but do express endogenous heavy chain genes[9]. This situation seemed paradoxical because the A-MuLV transformants were thought to represent the precursors of the cells that gave rise to the hybridomas. It now seems likely that in the transgenic M54 mice the A-MuLV transformants represent the conventional B-cell precursors that are unable to mature while the hybridomas mainly represent the CD5 (Ly-1) B-cell population. Other anomalies in the immunoglobulin repertoire of the transgenic mice, particularly the usage of a restricted set of V_H genes for the synthesis of antibodies by transgenic B cell hybridomas[9], may also be a consequence of the predominance of CD5 (Ly-1) B cells in these animals but interpretations must await more detailed analysis.

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