Expression of major histocompatibility complex (MHC) class II molecules is developmentally regulated and lineage dependent. Their role in hematopoiesis is not well defined. Previous studies in a canine model showed that dogs given 920 cGy of total body irradiation, transplanted with autologous marrow, and treated with anti-MHC class II monoclonal antibody (MoAb) immediately posttransplant experience only a transient granulocyte recovery that was followed by graft failure. In the present study, the effect of anti-MHC class II MoAbs on canine in vitro hematopoiesis was investigated. Anti-MHC class II MoAb H81.9 or B1F6 (both recognizing nonpolymorphic determinants) had no inhibitory effect when added directly to colony-forming unit–granulocyte-macrophage (CFU-GM) grown in agar. However, the addition of intact MoAb or as F(ab') fragments to long-term marrow cultures (LTMCs) resulted in a dose-dependent inhibition of the generation of CFU-GM among nonadherent cells. Inhibition was most profound with MoAb added at the time of initiation of culture. However, even if MoAb was added 3 weeks after recharging LTMCs, CFU-GM generation rapidly decreased. In addition, the number of adherent cells in LTMCs decreased; predominantly fibroblast-like cells with prominent cytoplasmic vacuolization remained. Acridine orange/ethidium bromide staining and TdT-mediated deoxyuridine triphosphate–digoxigenin nick end labeling (TUNEL) tests showed increased in the proportion of apoptotic cells in both the nonadherent and adherent compartments. Binding of anti-MHC class II MoAb to unfractionated marrow cells resulted in an increase in free (Ca++) and no changes in tyrosine phosphorylation pattern were observed. The addition of stem cell factor (SCF), but not granulocyte colony-stimulating factor or granulocyte-macrophage colony-stimulating factor, to LTMCs prevented apoptosis, and the generation of CFU-GM was indistinguishable from controls. Similarly, a supportive adherent layer was maintained. Thus, anti-MHC class II MoAbs interfere with hematopoiesis both in vitro and in vivo. The mechanism involves programmed cell death in subpopulations of adherent and nonadherent cells. Inhibition of hematopoiesis is abrogated by exogenous SCF.

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Table 1. Addition of MoAb to CFU-GM Assay

<table>
<thead>
<tr>
<th>Experiment</th>
<th>MoAb Added to Assay*</th>
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<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>116 ± 12</td>
</tr>
<tr>
<td>2</td>
<td>278 ± 21</td>
</tr>
</tbody>
</table>

* Marrow-derived mononuclear cells (1 x 10^5) were plated per culture; each assay was performed in triplicate and colonies were counted on day 14. MoAb concentrations were those found to be effective in long-term cultures, H81.9 at 10 μg/mL and B1F6 at 100 μg/mL.

† Cells were incubated at 4°C with H81.9 for 1 hour, washed and then incubated with complement (C') for 1 hour, washed, and plated.

This support, fresh marrow cells recharged onto this layer are able to generate mature hematopoietic cells for extended periods of time. We have used such a system in an attempt to define the mechanisms by which anti-MHC class II MoAbs may interfere with hematopoiesis, and show that these MoAbs inhibit hematopoiesis from nonadherent cells in LTMCs and morphologically alter the adherent layer. MHC class II-mediated inhibition is abrogated by the addition of exogenous c-kit ligand or stem cell factor (SCF).

MATERIALS AND METHODS

MoAbs

MoAb H81.9.F(ab')2 (H81.9; IgG2a), generated against mouse MHC class II antigens, is cross-reactive with human HLA-DR and canine

![Graphs](A) H81.9  
![Graphs](B) H81.9 F(ab')2  
![Graphs](C) B1F6  
![Graphs](D) G3G6

Fig 1. Dose/response effect of anti-MHC class II MoAbs on the generation of CFU-GM from LTMCs. LTMCs, after recharging, received one of the following MoAbs: (A) MoAb H81.9, (B) MoAb H81.9 F(ab')2, (C) MoAb B1F6, and (D) G3G6. Results are expressed as percentage of colony formation ± SD in control cultures. Absolute numbers for CFU-GM in control cultures at weeks 1, 2, 3, and 4 were (A) 123 ± 13, 72 ± 4, 31 ± 4, and 16 ± 2; (B) 90 ± 3, 62 ± 4, 38 ± 4, and 18 ± 2; (C) 99 ± 5, 52 ± 6, 36 ± 2, 22 ± 1 and 11 ± 2; and (D) 99 ± 9, 52 ± 5, 36 ± 8, and 22 ± 4, respectively.
MHC class II framework determinants. B1F6 (IgG2a) recognizes canine MHC class II framework determinants and is cross-reactive with HLA-DR and -DP. Fragments of H81.9 were obtained by digestion with pepsin and purification over protein-A columns as described. MoAb G3G6 (IgG2a), specific for the human platelet-associated glycoprotein Iib/IIa (C. Badger, unpublished observations, 1994) and nonreactive with canine cells, was used as control. MoAbs were purified from murine ascites or obtained from supernatant of hybridoma grown in the Fred Hutchinson Cancer Research Center bioproduction facility. The MoAbs were characterized by standard techniques and shown to be free of mycoplasma contamination as described.

LTMCs

Bone marrow (BM) aspirates were obtained from the humerus of anesthetized normal dogs, and LTMCs were established as described. Briefly, mononuclear marrow cells (MNCs; 2 × 10^6) were cultured in 25-cm² tissue culture flasks (Costar, Cambridge, MA) in RPMI-1640 medium (MA Bioproducts, Walkerville, MD), supplemented with 20% prescreened heat-inactivated horse serum, 10⁻⁷ mol/L hydrocortisone-21 phosphate (Sigma Chemical Co, St Louis, MO), 1% nonessential amino acids, 1% pyruvate, 2% glutamine, and 1% penicillin-streptomycin. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂ in air. After 1 week, nonadherent cells were removed, and half of the spent medium plus an equal volume of fresh medium and freshly aspirated autologous marrow buffy coat cells were recharged onto the adherent layers. Anti-MHC class II MoAb was added to flasks either upon initiation of culture or at the time of recharging. In some experiments, MoAb was added to cultures 6 days after recharging, ie, 24 hours before assay- ing nonadherent cells for colony formation in semisolid agar. Starting 1 week after recharging, nonadherent cells were procured at weekly intervals, pelleted, and counted, and aliquots were assayed for CFU-GM. The remaining cells were returned to the long-term culture flasks with spent medium and fresh medium mixed 1:1.

**CFU-GM Assay**

CFU-GM assays were performed with nonadherent cells from LTMCs and in additional experiments with fresh marrow cells. Nonadherent cells from LTMCs were assayed for CFU-GM as described. MNCs, 7.5 × 10⁶, were cultured for 14 days at 37°C in a humidified atmosphere of 5% CO₂ in air in 35-mm Petri dishes containing 2 mL of agar medium. The agar medium consisted of an equal volume mixture of 0.6% (wt/vol) Bacto agar (Difco, Detroit, MI) and double-strength Dulbecco’s modified Eagle’s medium (GIBCO, Grand Island, NY) containing 40% (vol/vol) heat-inactivated prescreened human AB plasma. Three replicate cultures per test were assayed. The morphology of cells in the colonies was determined on cytospin preparations of individually picked colonies stained according to Wright-Giemsa (Sigma, St Louis, MO). In addition, fresh marrow MNCs, either unmanipulated or incubated with MoAb for 30 or 60 minutes before being placed in culture, were assayed for CFU-GM.

**Hematopoietic Growth Factors**

Recombinant canine c-kit ligand (stem cell factor; rcSCF), rc granulocyte-macrophage colony-stimulating factor (rcGM-CSF), and rc granulocyte colony-stimulating factor (rcG-CSF) were provided by Amgen Inc (Thousand Oaks, CA). These factors were used at concentrations determined in ancillary studies: SCF at 1 to 500 ng/mL, GM-CSF at 10 to 100 ng/mL, and G-CSF at 100 to 500 ng/mL. In various LTMC experiments, either one of these factors was added alone or in conjunction with anti-MHC class II MoAb with the aim of determining whether the MoAb-mediated effect was abrogated.

**Morphologic Studies on LTMC**

**Light microscopy (LM).** In addition to standard culture flasks, LTMCs were established on chamber slides in flasks with 1-cm² wells (Nunc, Naperville, IL), as described. After 1 week in culture, the nonadherent cells were removed, and fresh autologous marrow buffy coat cells were recharged onto the adherent layers as described above, and anti-MHC class II MoAb was added at various time intervals. The adherent stromal layers were examined by LM. Additional slides were stained with acridine orange and ethidium bromide or examined by TdT-mediated deoxyuridine triphosphate-digoxigenin nick end labeling (TUNEL; Apop Tag Kit; OnCor Inc, Gaithersburg, MD) test to examine for apoptotic changes (see below).

**Electron microscopy.** Canine marrow MNCs were procured as described on chamber slides in flasks with 1-cm² wells and cultured in the presence of anti-MHC class II MoAbs. Stromal cells
Fig 3. Loss of adherent stromal layer in MoAb H81.9-treated cultures. MoAb H81.9 (10 μg/mL) was added either alone or along with SCF to flasks (1-cm² wells) after recharging. Typical areas of each culture are shown (×10 or ×20): (A) control, (B) MoAb H81.9, and (C) MoAb H81.9 plus SCF (10 ng/mL). Although H81.9-treated flasks show a loss of adherent cells relative to controls, the addition of SCF concurrently with H81.9 prevented adherent cell loss; in fact, adherent cells appeared larger and showed a tendency toward cluster formation.

were fixed in Karnovsky’s/Karnovsky2, washed in 1% OsO₄ for 1 hour on ice, and then dehydrated in sequential ethanol series and embedded. The sections were placed on copper grids and stained with uranyl acetate and lead citrate, and examined on a JEOL 100 SX electron microscope.³³

Immunoprecipitation and Immunoblots

Cells were lysed on ice with NP-40 lysis buffer and centrifuged at 13,000g to remove insoluble material as described.³⁴ Immune complexes were collected on protein A-sepharose beads, washed, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis.³⁵ Immunoblots with anti-PLCγ1 and antiphosphotyrosine were performed and antibody binding detected using ¹²⁵I protein A and autoradiography as described.³⁶,³⁷

Measurement of Cytoplasmic Calcium Ion ([Ca²⁺]ᵢ) Concentration

Ca²⁺ ion flux in indo-1 (Molecular Probes, Eugene, OR) loaded cells was measured with a model 50 HH12150 flow cytometer (Ortho, Westwood, MA) as described.³⁸,³⁹ Briefly, canine mononuclear cells were loaded with indo-1 acetoxymethyl ester by incubation for 5 minutes at 37°C, followed by incubation on ice at 4°C. Cells were then washed and resuspended in fresh medium and stored in the dark on ice until analysis. The modification of maintaining cells at 4°C rather than 37°C or room temperature, as is customary with

Fig 4. Electron microscopic appearance of stromal layer. Cells were maintained in complete medium with (B) or without (A) addition of MoAb H81.9 at 10 μg/mL. Cultures were photographed 7 days after MoAb addition. After MoAb treatment, there was an increase in fibrotic bands and vesiculation in the cytoplasm.
MHC CLASS II–MEDIATED INHIBITION OF HEMATOPOIESIS

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Fig 5. Effect of rcSCF, rcG-CSF, or rcGM-CSF on the generation of CFU-GM in MoAb H81.9-treated LTMCs. LTMCs were cultured in complete medium and treated as indicated. (A) MoAb H81.9 (10 μg/mL) alone, or MoAb H81.9 plus SCF (10 ng/mL). G-CSF (100 ng/mL), or GM-CSF (10 ng/mL) were added at the time of recharging. (B) LTMCs were performed in the presence of MoAb H81.9 (10 μg/mL) plus SCF (at 1, 5, 10, 100, or 500 ng/mL) both added at the time of recharging. Colony formation from nonadherent cells was as follows: (A) 87 ± 7, 55 ± 4, 35 ± 2, 20 ± 3, and 14 ± 1; (B) 116 ± 12, 76 ± 7, 48 ± 3, 19 ± 2, and 9 ± 2.

Human cells, was necessary to prevent rapid turnover and leakage of indo-1 from cells. For each assay, indo-1 loaded cells were diluted to 1 × 10⁶/mL with medium, equilibrated at 37°C in a waterbath, and analyzed by flow cytometry. Histograms were analyzed in regard to the mean indo-1 violet/blue fluorescence ratio as a function of time, and the percentages of cells with a particular indo-1 ratio above the mean for control cells was determined.

Microscopic Determination of Apoptosis in Nonadherent and Adherent Cells of LTMCs

Viability of nonadherent and adherent cells was determined using trypan blue dye exclusion and acridine orange/ethidium bromide staining. Cell morphology was evaluated on May-Grünewald-Giemsa–stained cytocentrifuged cell preparations. Apoptotic cellular changes were identified as described considering cell membrane alterations, chromatin condensation, and nuclear fragmentation.

In addition, the TUNEL test was applied to anti-MHC class II MoAb-treated nonadherent and adherent cells. Cultured nonadherent cells and adherent cells detached by treatment with trypsin (0.05%; 5 minutes) were centrifuged for 4 minutes at 400g at room temperature. The supernatant was discarded, and the pellet was resuspended in the remaining medium. Four percent formalin was added to the suspension. Drops of the cell suspension were placed on slides precoated with 0.01% poly-L-lysine and air dried. TdT labeling was performed as described by Gavrieli et al., except that peroxidase was developed with diaminobenzene. Slides were counterstained with methyl green.

Flow Cytometric Analysis of Apoptosis in LTMCs

To further quantitate anti-MHC class II MoAb-mediated apoptosis, a flow cytometric analysis of propidium iodide (PI)-stained cells was used with modifications as described. After various incubation times after recharging in LTMC, nonadherent and adherent cells were washed and pelleted, and the pellet gently resuspended in 1 mL hypotonic PI solution (Sigma p1304, 100 μg/mL; 0.1% sodium citrate, 0.1% Triton X-100; Ribonuclease A: Sigma R 4875, 0.1 mg/mL). Samples were allowed to equilibrate for at least 1 hour in the dark before analysis. Fluorescence analysis of individual nuclei was performed with the use of a FACScan flow cytometer equipped with an argon laser at 488 nm and 250 mW light output and lysis II software (Becton Dickinson, San Jose, CA). The fluorescence intensity from cell nuclei stained with PI is proportional to the cellular DNA content. Events (10 × 10⁴) were collected, stored, and analyzed by Multicycle software developed by Dr P. Rabinovitch (University of Washington, Seattle, WA).

Transmission Electron Microscopy of Apoptosis in LTMCs

Cultured nonadherent and adherent cells were collected by scraping with Cell Scraper (Baxter, McGaw Park, IL) in the presence of MoAb H81.9 alone or MoAb H81.9 plus SCF, and washed in phosphate-buffered saline (PBS). After washing, the cells were fixed

Fig 6. Immunoblot of marrow mononuclear cell lysates with anti-phosphotyrosine. Cells were exposed to various MoAbs for 2 or 5 minutes, and processed as described under Materials and Methods. No change in phosphorylation pattern was observed. Avidin, avidin control. The abbreviations represent various anti-MHC class II MoAbs: H, H81.9; B, B1F6; Ca, Ca1.41; Fab'1, fragments of H81.9; HB, HB10a; p4, p4.1.
MoAbs
In Vitro Hematopoiesis is Inhibited by Anti-MHC Class II MoAbs

Incubation of MNCs with MoAb H81.9 or B1F6 for 30 minutes or 1 hour before being placed in culture did not affect CFU-GM colony formation; only pretreatment of cells with MoAb plus complement reduced the number of CFU-GM (Table 1). However, if MoAb [intact or as F(ab')2 fragments] was added to LTMCs, the ability of nonadherent cells to form CFU-GM was inhibited in a dose-dependent fashion (Fig 1). Inhibition was most profound with MoAb added at the time of initiation of cultures and less so when MoAb was added later (Fig 2). Considering the possibility that the early presence of MoAb would not allow for the establishment of a functional adherent layer and normal contact to nonadherent cells, an adherent layer was established, recharged with nonadherent cells 1 week later, and cultures were left unperturbed for 2 weeks before H81.9 (10 µg/ml) was added. Beginning again 1 week later, nonadherent cells were assayed for CFU-GM. As with the earlier addition of MoAb, CFU-GM formation decreased to unmeasurable numbers by 3 weeks (not shown).

Anti-MHC Class II MoAb-Mediated Loss of Adherent Layers

As shown in Fig 3, in the presence of MoAb H81.9 there was a progressive decrease in the density of adherent cells in LTMCs. By light and electron microscopic examination, remaining cells showed fibroblastoid characteristics. Furthermore, adherent cells in MoAb-treated cultures contained more prominent cytoplasmatic vesicles than did cells in nontreated cultures (Fig 4).

Effect of Recombinant Growth Factor on Anti-MHC Class II MoAb-Mediated Inhibition of LTMCs

Previous studies had shown that dogs treated with MoAb H81.9 were rescued from graft failure if treated with exogenous SCF concurrently with MoAb administration, even though the kinetics of hematopoietic recovery were not completely normal.\(^{43}\) Therefore, we attempted to characterize a comparable effect of SCF (or other growth factors) in vitro. Three canine factors available in recombinant form (G-CSF, GM-CSF, and SCF)\(^{44,45}\) were tested (Fig 5). The presence of exogenous G-CSF or GM-CSF in MoAb-treated cultures had no recognizable effect. However, SCF at 10 ng/ml or higher completely prevented a decrease in CFU-GM that were cultured from nonadherent cells. In addition, the adherent layer of SCF-treated cultures was largely maintained and showed a prominence of large fibroblast-like cells with a tendency to form clusters with nonadherent cells.

Anti-MHC Class II MoAb Treatment and Transmembrane Signaling

Anti-MHC class II MoAbs, somewhat dependent upon the recognized epitope, mediate transmembrane signals and trigger \(Ca^{2+}\) mobilization in lymphocytes.\(^{38,46}\) We hypothesized that similar events occur in canine marrow cells.

BM mononuclear cells were incubated with intact anti-MHC class II MoAb (H81.9, B1F6, CA1.41, HB10a, p4.1) alone or in combination or with F(ab')2 fragments of H81.9. After 2 and 5 minutes, samples were procured and assayed for tyrosine phosphorylation. No new protein bands and no significant changes in the intensity of preexisting proteins were detectable (Fig 6).

Next, marrow mononuclear cells, loaded with indo-1, were treated with biotin-conjugated anti-MHC class II MoAb. There was no detectable change in \([Ca^{2+}]\) with the MoAb alone; a moderate signal was observed after cross-linking of biotin-conjugated H81.9 with avidin (Fig 7). Thus, these results showed calcium signaling, but suggested that a pathway other than tyrosine phosphorylation was involved.

Apoptosis in LTMCs

Nonadherent and adherent cells from LTMCs were stained with acridine orange/ethidium bromide or alternatively subjected to the TUNEL method at various time points after addition of MoAb H81.9 to cultures. Results are illustrated in Fig 8. As expected, some apoptotic nuclei were seen even in control cultures. However, the proportion of cells showing apoptosis was increased in MoAb-treated LTMCs, both in the adherent and the nonadherent
populations. As shown in Fig 8, the addition of exogenous SCF to cultures significantly reduced the proportion of apoptotic cells in H81.9-treated cultures. Overall, slightly more cells survived in the nonadherent compartment than among adherent cells.

Nonadherent and adherent cells from LTMCs were also analyzed for DNA content by flow cytometry (Fig 9). Beginning at 3 to 4 hours, apoptotic cell nuclei were detectable. Apoptotic nuclei were seen among both adherent and nonadherent cells. The proportions varied slightly from experiment to experiment (ranging from 6% to 15%) and reached a plateau at 24 hours. The concurrent presence of exogenous SCF consistently reduced or prevented the development of apoptosis. As shown in Fig 10, the electronmicroscopic appearance of treated cells was typical for apoptosis.
DISCUSSION

Cellular components of both the immune system and the hematopoietic system are thought to be derived from the same stem cell, and MHC genes are expressed in both lymphoid and hematopoietic lineages. However, although the role of MHC molecules within the immune system is well defined, their functions on hematopoietic cells are less well understood. In particular, the function of class II molecules on hematopoietic cells has remained controversial. Expression is locus specific, lineage dependent, and developmentally regulated. We have previously shown that MHC class II antigens are expressed on canine hematopoietic cells that are required for sustained recovery after autologous marrow transplantation. Recently, we observed that dogs given marrow-ablative doses of total body irradiation (920 cGy) and infused with unmanipulated autologous marrow fail to recover normal hematopoiesis and die with marrow aplasia if treated with anti-MHC class II MoAb in the immediate posttransplant period (days 0 through 4). To further investigate these observations, the present in vitro studies were undertaken.

The addition of anti-MHC class II MoAb to LTMCs resulted in a dose-dependent decrease of CFU-GM precursors among nonadherent cells. This was of note because no significant effect of these MoAbs was observed when added directly to CFU-GM cultures in agar. Although it is possible that, in a semisolid medium, MoAb would not be freely available to the hematopoietic cells, these results suggest that anti-MHC class II MoAb may have a role in the regulation of hematopoiesis. The mechanism by which anti-MHC class II MoAb inhibits hematopoiesis is not yet clear, but it may involve direct effects on hematopoietic progenitor cells or indirect effects through modulation of cytokine production.

Fig 9. Increased apoptotic DNA in cells from H81.9-treated marrow cultures and prevention of apoptosis by exogenous SCF. Nonadherent (A) and adherent cells (B) were analyzed at 4 and 24 hours after the addition of H81.9 (middle panel) or H81.9 plus SCF (right panel). Results with cells from untreated cultures are shown in the left panel. The vertical axis indicates cell numbers, the horizontal axis cellular DNA content. The dotted line in each panel delineates the original data. The continuing lines delineate the cell cycle phases calculated by the Multicycle program; the apoptotic peak is shaded and the percentage of apoptotic nuclei indicated by the number in each panel.
available and CFU-GM would not be exposed to concentrations sufficient for inhibition, this is unlikely because even MoAb concentrations 20-fold higher than used in LTMCs did not result in direct CFU-GM inhibition, and preincubation of CFU-GM with MoAb failed to result in an inhibitory effect. Thus, these results suggest that the anti-MHC class II MoAb exerted its effect not directly on CFU-GM, but rather on a less mature precursor cell or, alternatively, on nonhematopoietic accessory cells. These cells would be expected to express class II molecules. Indeed, morphologic analyses in the present study showed that anti-MHC class II MoAb interfered with the development of a normal functional adherent layer, a finding in agreement with observations by Brühl et al who had shown that the presence of anti-HLA-DR and -DP MoAb prevented the formation of confluent adherent layers in human in vitro models. Those results were surprising because MHC class II antigens are generally thought to be expressed minimally or not at all. They are, nevertheless, consistent with the finding that at least subpopulations of canine marrow-derived stromal cells express class II. Those cells could support hematopoiesis directly, eg, by serving as anchor or by providing growth factors, or indirectly via the generation of signals necessary for other supportive cells.

Consistent with the notion that subpopulations of cells are affected by anti-MHC class II MoAb is the analysis of apoptosis, showing that only a proportion of cells, both adherent and nonadherent, was involved. Apoptosis has been observed in B lymphocytes exposed to MoAbs directed at MHC class II and could be prevented by interleukin-4. Binding of MoAb to class II antigens has also been shown to induce tyrosine phosphorylation and Ca²⁺ flux in T lymphocytes. Superantigen-induced apoptosis of class I T cells is at least partially mediated by early class II signals from tyrosine kinase activation, but is also dependent upon late adhesion through beta-2 integrins. Experiments on transmembrane signaling in the present study showed evidence for class II-mediated calcium signals, although no change in tyrosine phosphorylation was seen in whole cell lysates. The role of adhesion, clearly MHC class II inducible in B cells, remains to be determined in the present model.

Regardless of the pathway, MoAb-mediated apoptosis and inhibition of hematopoiesis were prevented by exogenous SCF. Williams et al first reported that growth factor withdrawal from hematopoietic cultures in vitro resulted in apoptosis, and others showed that apoptosis in human erythroid progenitors was prevented or reduced by SCF even in the absence of erythropoietin. However, there is no direct evidence that both MHC class II and c-kit-dependent signals are mediated through the same cell. In fact, semiquantitative dilution experiments with highly enriched human CD34⁺ cells assayed for colony formation in the absence or presence of MoAb H81.9 suggest that an accessory cell is involved in the MHC-mediated effect. Such a hypothesis is also consistent with observations in the present study: anti-MHC class II MoAb-triggered apoptotic cell death was present in adherent and nonadherent cells; however, the addition of SCF maintained viability predominantly among nonadherent cells. This could be readily explained by an effect of MoAbs on accessory cells in the adherent compartment, which serve as a source of SCF. Loss of those cells, in turn, would result in cell death among nonadherent cells. The addition of exogenous SCF would primarily affect SCF-dependent nonadherent cells and much less so the adherent layer, although the SCF receptor c-kit appears to be expressed on certain stromal cells. Such a scenario would also accommodate in vivo observations: while the administration of SCF abrogated marrow failure in H81.9-treated dogs, a secondary decrease in granulocytes after the transient initial recovery was not completely prevented. These kinetics are consistent with recovery from a small pool of surviving stem cells rather than complete prevention of H81.9-induced damage.

Interestingly, the addition of G-CSF or GM-CSF to LTMCs failed to show any effect on stromal cells or hematopoiesis in the presence of anti-MHC class II MoAbs, suggesting that to override inhibition of hematopoiesis by anti-MHC class II MoAb, an early acting viability maintaining
factor such as SCF is required. If MoAb-mediated inhibition of hematopoiesis involves downregulation of growth factors, G-CSF and GM-CSF are unlikely candidates. In fact, preliminary data described elsewhere are consistent with this interpretation insofar as, at least at the mRNA level, G-CSF is not affected by anti-class II treatment, and GM-CSF is upregulated. However, stromal cells are also a major source of SCF, and particularly under stress conditions, optimum support of hematopoiesis by the stromal layer is required. Neta et al. have recently shown in mice that postirradiation hematopoietic recovery is impaired by treatment with anti-SCF antibody. Similarly, we have shown that neutralization of SCF in LTMCs using a specific MoAb results in exhaustion of hematopoiesis.

In conclusion, anti-MHC class II-mediated inhibition of hematopoiesis and abrogation by the c-kit ligand SCF involves a complex mechanism. Programmed cell death represents one component. Additional factors include stromal cell dysfunction and dysregulation of growth factors.

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