The Pattern of HLA-DR and HLA-DQ Antigen Expression on Clonable Subpopulations of Human Myeloid Progenitor Cells

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Three subpopulations of human myeloid progenitor cells (CFU-GM) can be distinguished by differences in their kinetics of development: the liquid phase pre-CFU-GM, the day 14 CFU-GM, and the day 7 CFU-GM. The relative cell membrane densities of the HLA-DR and HLA-DQ antigens expressed by these subpopulations was investigated by comparing the amount of antibody required to deplete bone marrow cell preparations of each cell type. Three separate approaches were used—complement (C') cytotoxicity, antiglobulin/C'-cytotoxicity and immune rosette depletion. Similar results were obtained for all three procedures, although the latter two gave a tenfold greater sensitivity over the standard C'-cytotoxicity method. At saturating anti-HLA-DR antibody concentrations, 85% to 95% of cells within the three myeloid subpopulations were found to express HLA-DR antigens. However, the relative amount of HLA-DR expressed by these subpopulations increased from the pre-CFU-GM to the day 7 CFU-GM. The expression of HLA-DQ antigens was considerably lower and could only be detected by using the more sensitive procedures. Only 50% of day 7 and 14 CFU-GM progenitor cells expressed detectable HLA-DQ antigens, whereas a greater proportion (80%) of the pre-CFU-GM were HLA-DQ positive. The pattern of HLA-DQ expression on these clonable precursors was quite distinct and opposite to the cell membrane density of the HLA-DR antigens. Because these three progenitor cell populations are thought to be linked in differentiation sequence, these results provide indirect support for the hypothesis that HLA class II antigens are implicated in regulatory mechanisms during normal myeloid cell differentiation.

The human major histocompatibility complex (MHC) codes for three distinct class II antigens, the HLA-DR, DQ (DC), and DP (SB) molecules, which are known to function as regulators in the immune response. These membrane antigens have a restricted cell distribution, being expressed predominantly by B lymphocytes, monocytes, and activated T lymphocytes. It is therefore of interest that hemopoietic progenitor cells of the myeloid and erythroid lineages also express at least one type of class II antigen at their cell membranes. These antigens are not found on the mature myeloid and erythroid cells, thus raising the intriguing possibility that class II antigens may also function as regulators of early events during hemopoiesis. Indeed, it has been shown that activated T lymphocyte-mediated inhibition of the more immature, clonal erythroid progenitors (BFU-E) is HLA-DR restricted. Furthermore, the expression of class II antigens on myeloid colony forming cells (CFU-GM) coincides with the ability of prostaglandin E and acidic isoferritins to inhibit in vitro colony formation.

Human myeloid progenitor cells which form colonies of mature progeny in cell culture can be divided into at least three subpopulations based on differences in physical properties, response to stimuli, and kinetics of development. This last characteristic is the one most commonly used to distinguish between these immature cell types, which are thought to be linked in a differentiation sequence. Thus, the pre-CFU-GM are the more immature progenitors which give rise to the day 14 CFU-GM, which in turn generate the more mature day 7 CFU-GM. The expression of HLA-DR antigens on these subpopulations of CFU-GM progenitor cells has been confirmed by several investigators, although some discrepancies exist. A number of investigators have demonstrated that 90% or more of the CFU-GM progenitor cell population express HLA-DR antigens, whereas others have reported that only 50% are HLA-DR positive. Similar inconsistencies have occurred for the more immature progenitor cells, the pre-CFU-GM, and the mixed CFU-GEMM colonies.

More recently, the pattern of HLA-DQ and DP antigen expression on hemopoietic progenitor cells has been studied. Compared with HLA-DR expression, HLA-DP molecules are only detected on a proportion of progenitor cells, whereas HLA-DQ molecules are expressed at very low levels, if at all.

In studying the patterns of class II antigen expression on hemopoietic progenitor cells, most reports have used antibodies at saturating levels. However, by comparing the amount of antibody required to deplete the different CFU-GM progenitor cell populations, information concerning the relative membrane densities of the antigen can be gained. This in turn may provide insight to the sequence of events in myeloid cell differentiation. Using this approach with a rabbit polyclonal anti-class II antigen serum, Moore et al found that the pre-CFU-GM progenitor cell population expressed the greatest abundance of class II antigen, followed by the day 14 CFU-GM, whereas the day 7 CFU-GM expressed the least. In contrast, Broxmeyer, using a monoclonal antibody (mcAb) to class II antigens, showed the reverse pattern—the pre-CFU-GM progenitor cell population appeared not to express class II antigens, whereas the day 7 CFU-GM expressed the most.

In this present study, we have established the titration curves of several anti-class II antigen mcAbs against the day 7 and 14 CFU-GM and pre-CFU-GM progenitor cells. Thus, using two anti-HLA-DR mcAbs, the pattern of expression reported by Broxmeyer was confirmed—the only difference being that a higher proportion (85% to 95%) of
progenitor cells from the three myeloid subpopulations expressed HLA-DR. By using sensitive procedures, HLA-DQ antigens were detected on a proportion of all three subpopulations. However, the cell membrane density of the HLA-DQ antigens was found to decline with cell maturation, which is exactly the reverse of the pattern of HLA-DR expression.

**MATERIALS AND METHODS**

*Antisera.* The B5.1 and F5C9 mcAbs have been described elsewhere. The B5.1 mAb is of the IgG2a subclass, and binds C'; it recognizes a monomorphic epitope expressed on DR molecules (personal communication, M. Steel, Co-ordinator, First International Workshop on Monoclonal Antibodies to Human MHC Class II Antigens, Edinburgh, Aug 28–Sept 2, 1983). The F5C9 mAb is of the IgG2a subclass, binds C' and recognizes an epitope expressed elsewhere. The B5.1 and F5C9 mcAbs have been described elsewhere.22 The B5.1 mAb is of the IgG2a subclass, and binds C'; it recognizes a monomorphic epitope expressed on DR molecules (personal communication, M. Steel, personal communication). High titred F5C9 and B5.1 antisera obtained from hybridoma-bearing mice were used in this present study. Another monoclonal anti-HLA class II mAb NEI-011 (also called 7.23) was purchased from New England Nuclear, Boston. It is of the IgG2b subclass, binds C, and is supplied as a protein A-affinity purified solution at 1 mg protein per milliliter (>95% homogeneity). McAbs used as controls were the monoclonal anti-HLA class I antibody, 246 B83 which is of the IgG2b subclass and binds C'; the 19A mAb (a gift of Dr H. Zola, Flinders Medical Centre, Bedford Park, South Australia) which recognizes a human common leukocyte determinant and is non-C'-fixing; and an anti-human T200 glycoprotein mAb, F2.3.25

The sheep anti-mouse immunoglobulin serum (SAMG) was a generous gift of Professor J. F. C. McKenzie, Department of Pathology, University of Melbourne. The immunoglobulin fraction of the antisera was isolated by ammonium sulfate precipitation using standard procedures.

*Bone Marrow Cells.* Sternal bone marrow specimens were obtained, with informed consent, from patients undergoing thoracic or cardiac surgery. Low-density marrow leukocytes were isolated on a Ficoll-Paque gradient (Pharmacia, Uppsala, Sweden), washed and resuspended in Dulbecco's Modified Eagles Medium (DME; Flow Laboratories, McLean, Va) containing 5% heat-inactivated bovine serum.

**Antibody-specific Cell Depletion Procedures**

All procedures used 17 x 100 mm plastic tubes (Falcon, Oxnard, Calif.).

*C'-dependent cytotoxicity.* Bone marrow leukocytes (1 x 10⁶) in 0.4 mL were mixed with an equal volume of antibody or normal mouse serum (NMS) at appropriate dilutions and were incubated for 30 minutes at room temperature (RT). The cells were washed and resuspended in 0.8 mL of a 1:2 dilution of rabbit C' (preselected for low nonspecific toxicity; Commonwealth Serum Laboratories, Melbourne, Australia) or heat-inactivated serum (for controls), and incubated a further 60 minutes at 37 °C. Controls included cells incubated with: no antibody (NMS) ± C'; anti-HLA class I mAb 246 B8 ± C' (positive C' control), and test antibody without C'.

*Anti-globulin/C'-cytotoxicity.* Bone marrow cells were treated as above except that, following the first antibody incubation, 1 mg SAMG (optimal concentration) was added. The cells were incubated for ten minutes at RT and washed and resuspended in rabbit C' as described. The non-C' binding 19A mAb was included as a control to assess the effectiveness of second antibody binding.

*Immune rosette depletion.* Bone marrow cells (1 x 10⁶) were incubated with antibody as above, washed three times, and resuspended in 0.5 mL of DME/5% serum. Controls included cells incubated with NMS or 246 B8 mAb. Rosette formation was performed as described elsewhere. Briefly, SAMG was coupled to sheep erythrocytes by chronic chloride, and 0.1 mL of a 2% erythrocyte suspension was added to the bone marrow cells. After a gentle centrifugation at 75 g for three minutes, the cell mixture was incubated for 30 minutes at RT to allow rosette formation. The cell pellet was then carefully resuspended in its supernatant and 0.4 mL of Ficoll-Paque was underlaid. Following centrifugation at 200 g for five minutes, the nonrosetted (interface) cells were collected.

For all procedures, the cells were given a final wash and divided for subsequent CFU-GM assays.

**Myeloid Progenitor Cell Colony Assays**

A 50% nonspecific loss of cells during the antibody-cell depletion procedures was assumed. Half the cells (~2.5 x 10⁹ total for control tubes) from each tube were assayed for pre-CFU-GM, while the remaining half were equally divided into six cultures (~4 x 10⁶ cells/culture) for day 7 and 14 CFU-GM.

*Day 7 and 14 CFU-GM.* For soft agar culture, DME containing the following supplements was used as the plating medium: 2 mmol/L of glutamine, 1 mmol/L of sodium pyruvate, 0.8% 50 x minimum essential amino acid mixture, 0.6% 100 x nonessential amino acid mixture, 16 μg/mL L-asparagine, 8 μg/mL L-serine, 0.6% vitamin mixture, 100 IU/mL penicillin, 100 μg/mL streptomycin, 0.12% sodium bicarbonate (all from Flow), 10⁻³ mol/L of 2-mercaptoethanol, 15% fetal calf serum (FCS, Commonwealth Serum Laboratories) and 0.3% bovaglar (Difco, Detroit). The cells were diluted in 6.5 mL of plating medium, containing an optimal concentration of colony-stimulating activity (CSA), and then equally divided into six 35-mm plastic culture dishes (Kayline, Adelaide, South Australia). The cultures were incubated at 37 °C in a humidified 10% CO₂ atmosphere; three plates were scored for colonies (> 50 cells) after seven days, and the remaining three were scored after 14 days of incubation.

*Pre-CFU-GM.* Pre-CFU-GM progenitor cells can be defined by their ability to give rise to CFU-GM following incubation in liquid suspension culture. In 17 x 100 mm Falcon 2057 tubes, cells (~2.5 x 10⁶) were suspended in 1 mL of DME containing 15% FCS, 10⁻³ mol/L of 2-mercaptoethanol and 10% CSA. After five days of suspension culture (maximal CFU-GM expansion was found to occur after four to five days of liquid culture and then to decline), 0.3 mL of cells was taken and plated in triplicate CFU-GM soft-agar cultures containing CSA, as described above. The plates were incubated for a further seven days, and CFU-GM colonies were scored. Under these conditions, expansion of the CFU-GM numbers ranged from 160% to 224% (mean 182%) as compared with day 7 CFU-GM numbers obtained without prior liquid culture.

*Sources of CSA.* Conditioned medium (CM) from the human fibroblast cell line 56372829 was routinely used as a source of CSA. For the preparation of 5637-CM, cells were grown in DME/5% bovine serum containing Cytodex-3 beads (Pharmacia, Uppsala, Sweden). After five days of culture, the CM was pooled, concentrated fivefold, and dialysed against 5 vol of deionized-distilled water using an Amicon TCF Thin Channel System with a YM10 filter (Amicon, Lexington, Mass). Following sterilization through a 0.2-μm filter, the 5637-CM was stored at -20 °C where it retained biological activity for at least six months. The optimum concentration of various batches of 5637-CM was found to be 5% to 10% for cultures containing 5 x 10⁶ cells per milliliter (day 7 CFU-GM numbers, 52 ± 15, mean ± SEM, n = 40). For some experiments, as indicated, CM from the human macrophage cell line GCT28 (GIBCO, Grand Island, NY) was used at a 10% final concentration.
**RESULTS**

**Pattern of expression of the HLA-DR antigens.** Figure 1A shows the results of a representative experiment (one of four separate experiments) in which the anti-HLA-DR mcAb B5.1 was titrated for inhibition of CFU-GM colony formation in the C'-dependent cytotoxicity assay. At high concentrations of mcAb (1:100 dilution), colony formation for the three CFU-GM progenitor cell compartments (day 7 and 14 CFU-GM and pre-CFU-GM) was inhibited by 85% to 95%, thus confirming the results of others.\(^2\)\(^{-}4\) However, at lower concentrations of mcAb, the sensitivities of the CFU-GM compartments diverged, with the day 7 CFU-GM as compared with the pre-CFU-GM. This was evidenced by the 50% titration points which show, in Fig 1A for example, that the day 7 CFU-GM titrated at a B5.1-antibody dilution of 2 \times 10^{-4}, the day 14 CFU-GM at 10^{-4}, whereas the pre-CFU-GM had the lowest titer at 0.3 \times 10^{-4}. Thus, a tenfold increase (range 5 to 15) in the expression of HLA-DR antigens was demonstrated for the day 7 CFU-GM as compared with the pre-CFU-GM. This pattern of HLA-DR expression is similar to that described by Broxmeyer,\(^6\) the only difference being in the proportion of progenitor cells expressing detectable HLA-DR antigens at saturating antibody levels.

![Fig 1](https://example.com/fig1)

**Table 1. Comparison of Sensitivities of Three Assay Procedures Used to Determine Expression of HLA-DR Antigens on Day 7 CFU-GM Progenitor Cells**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>C'-cytotoxicity</th>
<th>Anti-globulin/C'-cytotoxicity</th>
<th>Rosette Depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10(^2)</td>
<td>95 ± 1</td>
<td>97 ± 2</td>
<td>94 ± 1</td>
</tr>
<tr>
<td>1:10(^3)</td>
<td>83 ± 2</td>
<td>95 ± 2</td>
<td>96 ± 2</td>
</tr>
<tr>
<td>1:10(^4)</td>
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<td>73 ± 3</td>
<td>85 ± 3</td>
</tr>
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<td>1:10(^5)</td>
<td>24 ± 5</td>
<td>59 ± 2</td>
<td>61 ± 3</td>
</tr>
<tr>
<td>1:10(^6)</td>
<td>8 ± 4</td>
<td>28 ± 4</td>
<td>33 ± 4</td>
</tr>
</tbody>
</table>

*Results are the mean ± SE of triplicate cultures.*
immune rosette depletion, were used. At high concentrations of F5C9 mAb, ~50% (range 42% to 72%) of day 7 and 14 CFU-GM were found to express HLA-DQ antigens, whereas an even greater proportion (75% to 85%) of the pre-CFU-GM were HLA-DQ antigen positive (Fig 3). Information from the antibody titration curves suggests that the level of HLA-DQ antigen expression decreases with increasing maturation such that the pre-CFU-GM progenitor cells express the greatest level, followed by the day 14 CFU-GM, whereas the day 7 CFU-GM progenitors express the least. Comparison of the 40% titration points, for example, indicates a 50-fold (range 25 to 200) decrease in HLA-DQ antigen expression by the day 7 CFU-GM relative to the pre-CFU-GM progenitors. This pattern of expression is the reverse of that found for the HLA-DR antigens.

Patterns of expression of non-HLA class II antigens. To show that technical manipulations were not accountable for the patterns of HLA-DR and DQ antigen expression presented here, titration curves were established using two mAbs which recognize different and unrelated cell membrane antigens. The 246 B8 mAb recognizes a monomorphic epitope on HLA class I antigens, whereas the F2.5 mAb recognizes the human homologue of the murine T200 glycoprotein (or leukocyte-common antigen). For both antibodies, no significant difference was observed between the titration curves for day 7 and day 14 CFU-GM progenitor cells (Fig 4). Thus, unlike the HLA-DR and DQ molecules, the expression of the HLA class I and T200 antigens is static during this period of myeloid cell differentiation.

**DISCUSSION**

In this study, the cell membrane density of the HLA-DR antigens was shown to increase with myeloid progenitor cell maturation. An average tenfold increase in the expression of HLA-DR antigens by day 7 CFU-GM progenitor cells as compared with the pre-CFU-GM was demonstrated. These results confirm those of Broxmeyer, although they vary with respect to the observed maximum levels of HLA-DR antigen expression. Broxmeyer found, even at high anti-HLA-DR mAb concentrations, that only 50% of the day 7 and 14 CFU-GM progenitors expressed the antigen, whereas the pre-CFU-GM appeared not to express HLA-DR at all. Our results show that 85% to 95% of cells from the three myeloid progenitor cell compartments studied express HLA-DR antigens. Presumably the variance in results between those reported here and those reported by Broxmeyer can be attributed to the different sources of complement used, an effect recently illustrated by Falkenburg et al, who showed...
that hemopoietic progenitor cells differ in their sensitivities to complement. However, the use of complement-dependent procedures in this report does not seem to have biased the results in any way, because similar results were obtained using a noncomplement procedure, immune rosette depletion.

The demonstrable increase in the level of HLA-DR antigen expression during CFU-GM progenitor cell maturation supports the hypothesis that these antigens have a biological function in hemopoiesis. The exact nature of such a role remains unknown. An association between HLA-DR expression and modulation of myeloid differentiation by prostaglandin E and acidic isoferritins has been suggested. However, in contrast to our data and those of other investigators, these authors found that only 50% of the CFU-GM progenitor cells expressed HLA-DR; thus, the correlations observed must remain uncertain.

It could be argued that the observed increase in HLA-DR expression may not be unique but could be a common occurrence for cell membrane proteins expressed by the CFU-GM progenitor cells. Evidence against this, however, is that the expression of the separately regulated HLA class I and T200 glycoproteins remains constant during the three stages of myeloid cell differentiation studied. Furthermore, the expression of the HLA-DQ antigens was found to decline with cell maturation.

Of particular interest in this study was the demonstrated pattern of HLA-DQ antigen expression by myeloid progenitor cells. At saturating antibody levels, ~50% of the day 7 and 14 CFU-GM progenitor cells expressed detectable HLA-DQ antigens, whereas an even greater proportion (80%) of pre-CFU-GM progenitors were HLA-DQ positive. Comparison of the titration curves for the three cell populations indicated that the pre-CFU-GM expressed 50 times more HLA-DQ (range 25 to 200) than did the day 7 CFU-GM. This pattern of expression is the reverse to that found for the HLA-DR antigens (see Fig 5 for a diagrammatic representation).

These results could only be obtained when very sensitive procedures were used. The anti-HLA-DQ mAb F5C9 used in this study is strongly active in the C'-cytotoxicity assay when B lymphocytes are used as target cells. The fact that very little F5C9-binding could be detected on myeloid progenitor cells using a noncomplement procedure, immune rosette depletion, but their definition of a day 7 CFU-GM colony varied considerably from ours, and thus the results are not directly comparable.

We are currently pursuing the investigation of HLA-DQ antigens on hemopoietic cells. It is possible that the apparent decrease in HLA-DQ antigen expression may result from increased glycosylation of the molecule such that the F5C9 mAb can no longer recognize its reactive determinant. No data is available to suggest that this is the case. The biologic function of the HLA-DQ antigens on myeloid progenitor cells is unknown. However, in the immune system, HLA-DQ antigens predominantly modulate functions involving the production of suppressor and/or cytotoxic T lymphocytes, whereas the HLA-DR antigens are directly implicated in helper T lymphocyte function. It is therefore tempting to speculate that if similar roles are played by these antigens in hemopoiesis, the decrease in HLA-DQ and corresponding increase in HLA-DR expression seen during myeloid progenitor cell maturation reflect changes in regulatory mechanisms.

ACKNOWLEDGMENT

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