HUMAN HEMATOPOIETIC PROGENITOR CELLS IN LONG-TERM CULTURES EXPRESS HLA-DR ANTIGENS AND LACK HLA-DQ ANTIGENS

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The expression of class II determinants (Ia-like antigens) on human hematopoietic progenitor cells (HPC)[1] cultured in semisolid medium, has been extensively studied for CFU-GM (colony-forming unit precursor of granulocyte/macrophage) (1), BFU-E (burst-forming unit of erythroid cells; the immature erythroid committed progenitor cell) (1), CFU-E (colony-forming unit of erythroid cells; the relatively mature erythroid committed progenitor cell) (1), and CFU-GEMM (colony-forming unit of granulocyte/erythrocyte/macrophage/megakaryocyte; the multilineage HPC) (2). With both polyclonal and monoclonal antibodies (mAb), the expression of polymorphic and monomorphic HLA-DR determinants has been demonstrated on all HPC, using fluorescence-activated cell sorting (FACS) and complement-dependent cytotoxicity (CDC) assays (3–7), although some conflicting results have been published (4, 5) concerning the expression of HLA-DR determinants on CFU-E. On the other hand, HLA-DR-linked HLA-DQ (DC, MB, LB) determinants appear not to be expressed on human HPC in semisolid cultures (7–9). In mice, it has been reported (10, 11) that the pluripotential stem cell CFU of spleen do not express Ia antigens. Actually, I-A antigens (equivalent to human HLA-DQ) seem not to be expressed on CFU of spleen, whereas I-E antigens (equivalent to human HLA-DR) are (12). In dogs, no engraftment could be achieved after complete removal of Ia^+ cells from autologous grafts (13).

In man, there is no in vitro assay to determine the true pluripotent stem cell that is responsible for the hematopoietic reconstitution after transplantation of bone marrow cells into lethally irradiated recipients. However, from liquid long-term hematopoietic cell cultures, HPC can be cultured for many weeks (14). Thus, in this long-term culture system, a precursor cell responsible for the

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[1] *Abbreviations used in this paper:* BFU, burst-forming unit; CDC, complement-dependent cytotoxicity; CFU, colony-forming unit; E, erythroid; FACS, fluorescence-activated cell sorter; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; GEMM, granulocyte/erythrocyte/macrophage/megakaryocyte; GM, granulocyte/macrophage; HPC, hematopoietic progenitor cell; mAb, monoclonal antibody; α-MEM, α-modified Eagle's minimum essential medium.

sustained proliferation of committed progenitor cells may be analyzed. This precursor cell may be closely linked to the human true pluripotent stem cell.

In such a long-term culture system, Moore et al. (16) could not identify class II determinants on HPC responsible for the sustained proliferation of BFU-E and CFU-GM, although the precursor cell of CFU-GM appeared to express class II determinants. However, other investigators (17) found inhibition of HPC in a similar long-term culture system after treatment with anti-HLA-DR and complement. Keating et al. (18) could not detect class II determinants on precursor cells responsible for the proliferation of CFU-GM in a different (one-stage) long-term culture system, using one anti–class II antibody in a CDC assay, but inconclusive results were obtained using another class II antibody. In all these studies, only depletion techniques were used to identify these antigens on HPC in long-term culture systems. Previously (19), we showed that, using CDC assays only, antigenic determinants with weak expression on HPC can easily be overlooked.

Here, we report the expression of HLA-DR determinants and the absence of HLA-DQ determinants on precursor cells responsible for the sustained proliferation of CFU-GM, BFU-E, and CFU-GEMM in liquid long-term cultures, using both FACS and CDC assays.

Materials and Methods

Collection and Preparation of Bone Marrow Cells. Bone marrow cells were obtained, after informed consent, by aspiration from the posterior iliac crests of donors for bone marrow transplantation, and were collected in Hanks' balanced salt solution with 100 U/ml of preservative-free heparin. To establish stromal layers, red blood cells were lysed with an NH_4Cl buffer (0.155 M NH_4Cl, 0.01 M $KHCO_3$, 0.1 mM EDTA, pH 7.4) for 10 min at 0°C. The cell suspension was washed in RPMI 1640 with 5% fetal bovine serum (FBS) (Gibco Laboratories, Grand Island, NY) at 0°C, and resuspended in α-modified Eagle's minimal essential medium (α-MEM) (Flow Laboratories, Irvine, United Kingdom). For use in reinoculation, bone marrow cells were centrifuged over Ficoll-Isopaque (1.077 g/cm^3, 1,000 *g*, 20 min, 20°C), and the interphase was washed twice. These interphase cells were resuspended in a medium consisting of 70% RPMI 1640, 20% FBS, and 10% dimethylsulfoxide at 0°C. The cell suspensions were frozen in a computer-controlled freezer at a rate of 1°C/min from 0°C to −50°C, and at a rate of 4°C/min from −50°C to −90°C. The cells were then stored in liquid nitrogen. Before reinoculation, the cells were thawed for 1 min in a 37°C water bath, diluted in Hepes-buffered RPMI 1640 with 20% FBS at 0°C, and washed twice in the same medium at 0°C. The cells were then treated in a CDC assay, or separated using cell sorting with the FACS IV (Becton-Dickinson Immunocytochemistry Systems, Sunnyvale, CA), and reinoculated on the preestablished stromal layer.

Liquid Long-term Cultures (15). 10^7 bone marrow cells collected after red blood cell lysis were cultured in a fully humidified atmosphere of 33°C and 5% CO_2, in plastic culture flasks in 10 ml of medium consisting of Hepes-buffered α-MEM, with 25% fresh (frozen) ABO-compatible human heparin plasma and 10^{-6} M hydrocortisone. Once per week, half of the nonadherent cell suspension was removed, and 5 ml of fresh medium was added. After 4–5 wk, a stromal layer was established.

After treatment of cryopreserved autologous bone marrow mononuclear cells in a CDC assay or using FACS, these cells were inoculated on the preestablished stromal layers. The suspension of the day of reinoculation was called week 0. From weeks 1–6 after reinoculation, the nonadherent suspensions were demipopulated weekly, and 5 ml of fresh medium was added. The harvested cell suspension was cultured for CFU-GM (defined as aggregates of >20 granulocytic, monocytic, or eosinophilic cells), BFU-E (defined as bursts

of colonies consisting of hemoglobinized cells), and CFU-GEMM (defined as colonies containing at least both erythroid and myeloid cells (2, 7), in a medium consisting of 30% fresh (frozen) ABO-compatible human heparin plasma, 7.5% phytohemagglutinin-stimulated leukocyte–conditioned medium, 5% 10^{-5} M 2-mercaptoethanol, 5% deionized bovine serum albumin, 5% human transferrin, 5% Iscove's modified Dulbecco's medium, 1 U/ml erythropoietin (2.5%), and 40% methylcellulose 2.8% (at 37°C, in a fully humidified atmosphere with 5% CO_2). The numbers of HPC were counted on days 14–18.

Antibodies. mAb B8.11.2 (Malissen, Marseille, France) an IgG2b antibody exclusively reactive with HLA-DR molecules and not reactive with HLA-DQ determinants, was used in both CDC and in cell-sorting experiments. In CDC, the anti-DQwl antibody IIB.3, an IgG2b antibody (F. Koning, Leiden, The Netherlands) was used, and in cell-sorting experiments, the anti-DQwl antibody, Genox 3.53, an IgG1 antibody (Bodmer, London, United Kingdom) was used. Anti-DQwl antibodies were used only against bone marrow cells of $DQwl^+$ donors. All B cells of the $DQwl^+$ donors strongly reacted with the anti-DQwl antibodies (data not shown). All antibodies were used as diluted ascites (1:100).

Complement-dependent Cytotoxicity. 10^7 cryopreserved mononuclear bone marrow cells in 1 ml medium were incubated with an equal volume of mAb for 1 h at 20°C. Baby rabbit complement (prescreened batches) (Pel-Freez Biologicals, Rogers, AR) was then added to a final concentration of 20% in case of anti-HLA-DR antibodies, and 40% in case of anti-HLA-DQwl antibodies, which had been proven (7, 19) to be the highest complement concentration that was intrinsically nontoxic. After another 1-h incubation at 20°C, the cells were washed three times in RPMI 1640 plus 5% FBS, resuspended in α-MEM, and inoculated on the preestablished autologous stromal layers. As a control, 10^7 cells were incubated with antibody only, and reinoculated on an autologous stromal layer.

Fluorescence-activated Cell Sorting. Cryopreserved mononuclear cells at a concentration of 10^7 cells/ml were incubated with equal volumes of antibody for 1 h at 20°C, and washed three times in RPMI 1640 plus 5% FBS. Cells were then reincubated for 30 min at 20°C with fluorescein isothiocyanate (FITC)-labeled goat anti–mouse Ig (Nordic Immunology, Tilburg, the Netherlands), and washed twice. $3–10 \times 10^6$ cells were separated into a negative and a positive fraction using the Becton-Dickinson FACS-IV. For HLA-DR antibodies, the cut-off point was chosen at fluorescence intensity channel 120 (logarithmic scale). After incubation with irrelevant control antibodies, no HPC could be recovered from the positive channels 121–255. For HLA-DQ antibodies, the cut-off point was chosen at channel 60. After incubation of the cells with irrelevant control antibodies, >95% of the HPC were recovered from the negative fraction (channels 1–60). After sorting, the positive and negative fractions were inoculated on preestablished autologous stromal layers. As a control, an equal number of unseparated cells was reinoculated on an autologous stromal layer, thus determining the 100% growth value.

Calculations. In Figs. 1 and 3, the numbers of HPC are shown, corrected for demipopulation after week 1. In Figs. 2, 4, and 5, and in Table 1, the numbers of HPC cultured from the treated fractions from weeks 0–6 after reinoculation were expressed as percentage of the numbers of HPC cultured from the control suspension at that particular week.

The regeneration index was calculated by dividing the total number of HPC, corrected for demipopulation, obtained after reinoculation in weeks 1–6, by the number of HPC in the reinoculated suspension (week 0).

In Table II, the cumulative numbers of HPC after reinoculation (weeks 1–6) per treated fraction are expressed as percentage of the cumulative numbers of HPC cultured from the control suspensions (weeks 1–6), and compared with the numbers of HPC cultured from the reinoculated suspensions (week 0).

Results

The regeneration index was 2.38 ± 0.26 for CFU-GM, 1.00 ± 0.12 for BFU-E, and 1.87 ± 0.34 for CFU-GEMM (mean ± SE; 18 experiments), indicating sustained proliferation of HPC after reinoculation.

As shown in Fig. 1, elimination of nearly all proliferating HPC after reinoculation was observed after treatment of the bone marrow cell suspension with anti-HLA-DR antibodies and complement before reinoculation. There was no inhibition of proliferation of HPC after incubation with antibody or complement only (data not shown). Fig. 2 shows that, although anti-DR antibodies almost completely abolished the generation of HPC after reinoculation, treatment of the bone marrow cells with anti-HLA-DQw1 antibody IIB.3 and 40% complement before reinoculation did not result in a significant decrease in HPC proliferation. We used the highest complement concentration that was intrinsically nontoxic to show that HLA-DQ antigens are not expressed on these HPC, since it has

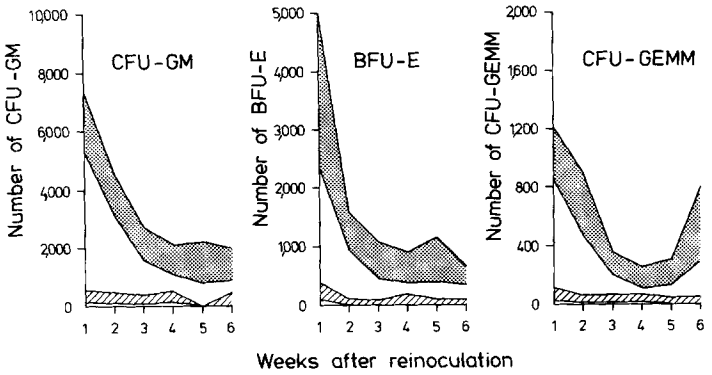

FIGURE 1. Recovery of proliferated HPC, corrected for demipopulation, after reinoculation of 10^7 cryopreserved autologous mononuclear bone marrow cells, treated with anti-HLA-DR, with or without addition of complement to a final concentration of 20%. Areas indicate mean ± SE of six experiments. □, anti-DR antibody only, ▨, anti-DR plus complement.

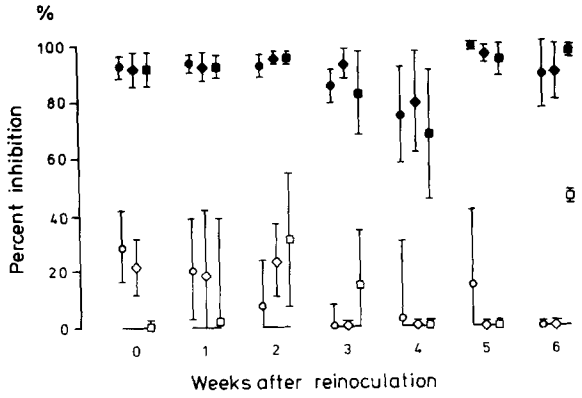

FIGURE 2. Inhibition of proliferated CFU-GM (○ and ●), BFU-E (◇ and ◆), and CFU-GEMM (□ and ■), after treatment of the reinoculated cell suspension with anti-HLA-DQ (open symbols; $n = 4$) and 40% baby rabbit complement (prescreened, intrinsically nontoxic), or with anti-HLA-DR (filled symbols; $n = 6$) and 20% complement, as compared to the control suspension, incubated with the antibody only. Bars indicate mean ± SE.

been shown (19) that antigens with low density on HPC can easily be overlooked when low complement concentrations are used in CDC assays. As shown in Fig. 3, after separation of the reinoculum into HLA-DR$^+$ and HLA-DR$^-$ fractions, using B8.11.2 mAb and cell sorting, the majority of CFU-GM, BFU-E, and CFU-GEMM were recovered from the HLA-DR$^+$ fractions during the 6 wk after reinoculation. <20% of the HPC could be recovered from the HLA-DR$^-$ reinoculum, as compared to the unseparated controls per week after reinoculation (Fig. 4). As shown in Fig. 5, hardly any HPC could be recovered from the HLA-DQwl$^+$ fractions after reinoculation of mononuclear bone marrow cells from HLA-DQwl$^+$ donors after cell sorting using the anti-HLA-DQwl antibody, Genox 3.53.

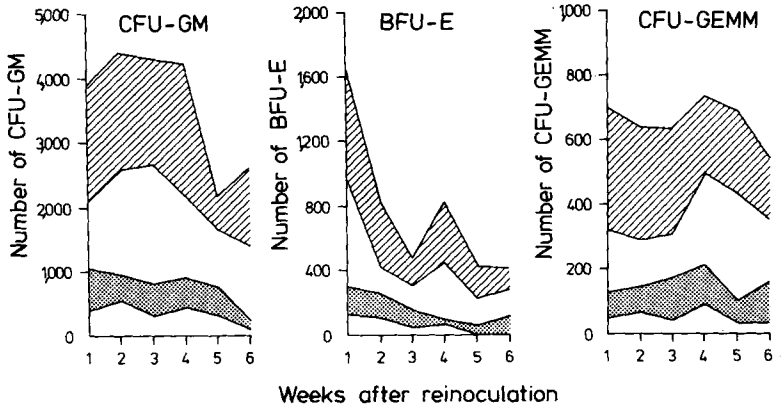

FIGURE 3. Recovery of proliferated hematopoietic progenitor cells, corrected for demipopulation, after reinoculation of 3 × 10^6 cryopreserved autologous mononuclear bone marrow cells, separated into an HLA-DR$^+$ and an HLA-DR$^-$ fraction, using FACS. Areas indicate mean ± SE of five experiments. □, DR$^-$ fraction; ▨, DR$^+$ fraction.

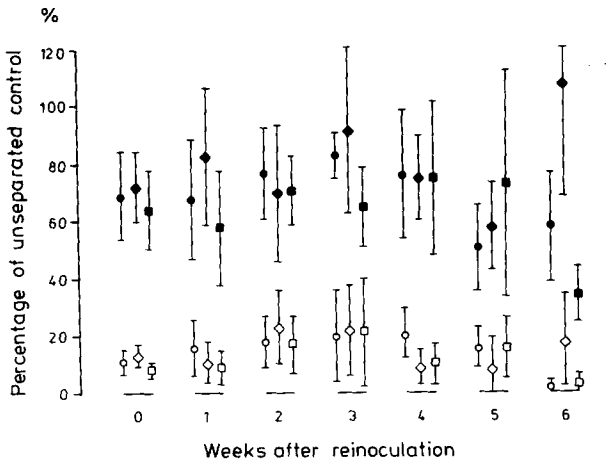

FIGURE 4. Recovery of proliferated CFU-GM (○ and ●), BFU-E (◇ and ◆), and CFU-GEMM (□ and ■) after cell sorting using anti-HLA-DR. Filled symbols represent the percentages of HPC in the positive fractions; open symbols those in the negative fractions, as compared to the unseparated control of that week. Bars indicate mean ± SE of five experiments, in case of week 6, three experiments.

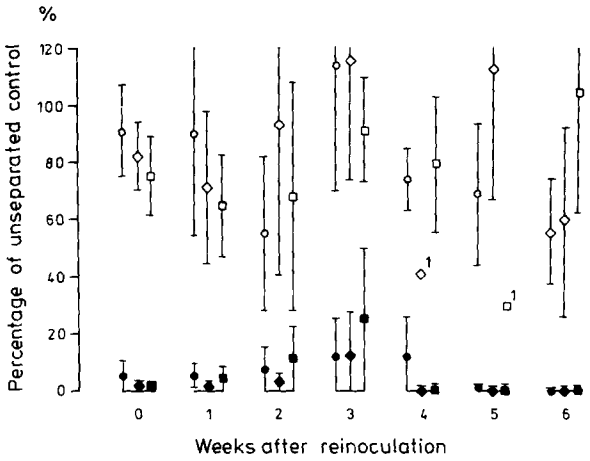

FIGURE 5. Recovery of proliferated CFU-GM (○ and ●), BFU-E (◇ and ◆), and CFU-GEMM (□ and ■) after cell sorting, using anti-HLA-DQwl and bone marrow cells of HLA-DQwl$^+$ donors. Filled symbols represent the percentages of HPC in the positive fractions; open symbols those in the negative fractions, as compared to the unseparated control of that week. Bars indicate mean ± SE of three experiments. Symbols with "1" superscript indicate one experiment; in the two other experiments, HPC from week 4 were destroyed by infection of the semisolid cultures.

To avoid the possibility that nonspecific differences in antibody binding would be responsible for these findings, the HLA-DR$^+$ fraction was defined as strongly positive fluorescence intensity (channels 121–255). In case of HLA-DQ antibodies, the HLA-DQ$^+$ fraction was defined as channels 61–255. When mAb with irrelevant specificity or the secondary FITC-labeled antibodies only were used, no HPC could be recovered from channels 121–255. No substantial difference in HPC recovery from the negative (channels 1–60) or positive (channels 61–255) fractions were found, no matter whether HLA-DQ antibodies or irrelevant control antibodies were used (data not shown).

To exclude the possibility that, in cell sorting experiments, HLA-DR$^+$ accessory cells present in the reinoculated bone marrow might have activated residual HPC present in the stromal layer before reinoculation, so that the HPC responsible for the proliferation after reinoculation were not actually obtained from the HLA-DR$^+$ fraction, we performed the cell sorting experiment described in Table I. Irradiation of the stromal layer with 3,000 rad before reinoculation did not affect the proliferation of HPC in the HLA-DR$^+$ and HLA-DR$^-$ fractions, nor in the unseparated control as compared to the nonirradiated control. Therefore, we conclude that the HPC responsible for the sustained proliferation of CFU-GM, BFU-E, and CFU-GEMM originated from the reinoculated HLA-DR$^+$ fraction.

In Table II, the cumulative numbers of class II–positive HPC recovered after reinoculation, expressed as percentages of the cumulative numbers of HPC in the control suspension, are compared with the percentages of class II–positive HPC in the suspension at the time of reinoculation. Using both FACS and CDC, the vast majority of HPC appeared to be HLA-DR$^+$ and HLA-DR$^-$. In fact, there were no substantial differences beween the percentages of class II–positive

TABLE 1

Influence of Irradiation of Stromal Layer on Recovery of HPC Reinoculated after FACS Separation of Bone Marrow Cells into HLA-DR$^+$ and HLA-DR$^-$ Fractions

HPC	Week	Absolute numbers of HPC (corrected for demipopulation)							
		Not reinoculated		HLA-DR$^-$		HLA-DR$^+$		Unseparated control	
		Unirradiated	Irradiated*	Unirradiated	Irradiated*	Unirradiated	Irradiated*	Unirradiated	Irradiated*
CFU-GM	1	104	0	127	209	1,346	1,573	2,784	3,328
	2	86	0	465	434	2,789	3,292	4,389	3,912
	3	231	0	1,260	856	1,389	884	2,081	2,677
	4	139	0	533	167	1,986	4,053	3,714	3,048
	5	375	0	483	150	1,815	3,123	2,627	3,079
	6	0	0	0	0	0	0	0	0
Total (weeks 1–6)		935	0	2,868	1,816	9,325	12,925	15,595	16,044
	1	92	0	87	127	711	637	2,007	1,741
	2	86	0	416	233	1,357	1,314	2,640	2,433
	3	33	0	285	342	245	397	425	586
	4	139	0	164	268	917	1,004	890	1,151
	5	0	0	138	75	435	213	438	80
	6	0	0	0	0	0	0	0	0
Total (weeks 1–6)		350	0	1,090	1,045	3,665	3,565	6,400	5,991
CFU-GEMM	1	46	0	51	31	292	353	735	666
	2	37	0	167	124	996	945	1,445	1,101
	3	66	0	346	86	245	137	446	418
	4	0	0	164	33	343	557	817	677
	5	0	0	70	75	218	284	438	163
	6	0	0	0	0	0	0	0	0
Total (weeks 1–6)		149	0	798	349	2,094	2,276	3,881	3,025

6×10^6 mononuclear bone marrow cells were separated into an HLA-DR$^-$ and an HLA-DR$^+$ fraction, using FACS (61% and 39%, respectively). Half of the sorted cells per fraction were reinoculated on an irradiated autologous stromal layer, and the other half on an unirradiated stromal layer. As a control, 3×10^6 unseparated cells were reinoculated on an irradiated stromal layer, and an equal number on an unirradiated stromal layer.

* Stromal layers were irradiated with 3,000 rad immediately before reinoculation.

HPC responsible for the sustained proliferation of HPC in long-term cultures and the percentages of class II–positive HPC cultured from the bone marrow suspension in semisolid medium before reinoculation. In the FACS experiments, the cut-off point was chosen at channel 120 to select only clearly positive cells, resulting in the allocation of up to 20% of HPC to the negative fraction. The regeneration indices of the negative and clearly HLA-DR$^+$ fractions were similar, indicating that the HPC in the HLA-DR$^-$ fraction did not have an increased proliferation potential as compared to the HLA-DR$^+$ fraction.

Discussion

Using CDC, our data clearly show that proliferation of HPC after reinoculation can be abolished by treatment of the bone marrow cells with anti-HLA-DR and

TABLE II

Comparison of Class II Expression on HPC Present in Bone Marrow at Time of Reinoculation with Class II Expression on HPC Responsible for Proliferation after Reinoculation

HPC subtype	HPC per fraction after separation (percentage of unseparated control)				Percent inhibition of CDC	
	DR^+ ($n = 5$)	DR^- ($n = 5$)	DQ^+ ($n = 3$)	DQ^- ($n = 3$)	Anti-DR + C (20%) ($n = 6$)	Anti-DQ + C (40%) ($n = 4$)
Present at time of reinoculation						
CFU-GM	68 ± 15*	11 ± 4	5 ± 5	91 ± 16	92 ± 4	29 ± 13
BFU-E	72 ± 12	13 ± 4	2 ± 1	82 ± 12	92 ± 6	22 ± 10
CFU-GEMM	64 ± 14	8 ± 2	1 ± 1	75 ± 14	92 ± 6	0 ± 2
Cumulative number harvested after reinoculation						
CFU-GM	62 ± 13	12 ± 5	7 ± 7	71 ± 23	91 ± 4	9 ± 14
BFU-E	76 ± 18	9 ± 3	4 ± 5	90 ± 31	93 ± 4	10 ± 18
CFU-GEMM	58 ± 9	9 ± 4	7 ± 8	80 ± 29	92 ± 3	22 ± 19

* Mean ± SE.

complement before reinoculation (Figs. 1 and 2). Although these results indicate that the precursor cells responsible for the sustained proliferation of HPC are $HLA\text{-}DR^+$, this phenomenon might also be explained by elimination of reinoculated $HLA\text{-}DR^+$ accessory cells required for the proliferation of HPC. To exclude this possibility, FACS experiments were performed. After incubation of the bone marrow cells with anti-HLA-DR, the suspension was separated into an $HLA\text{-}DR^-$ and $HLA\text{-}DR^+$ fraction, and reinoculated. The vast majority of the proliferated CFU-GM, BFU-E, and CFU-GEMM were cultured from the $HLA\text{-}DR^+$ fractions after reinoculation (Figs. 3 and 4), demonstrating that the precursor cells recovered from the reinoculated suspensions express the HLA-DR determinants themselves.

To further exclude the possibility that the proliferation of HPC after reinoculation was due to activation of residual HPC in the stromal layer by $HLA\text{-}DR^+$ accessory cells from the sorted DR^+ fraction, the previously established stromal layer was irradiated before reinoculation. Since this irradiation did not influence the results (Table I), the precursor cells responsible for the maintenance of HPC growth after reinoculation must have been recovered from the $HLA\text{-}DR^+$ fraction. Therefore, these precursor cells were $HLA\text{-}DR^+$ themselves.

On the other hand, anti-HLA-DQ antibodies, which are directed against another class II determinant closely linked to HLA-DR, did not react with precursor cells responsible for the proliferation of HPC in long-term cultures, neither in CDC nor in FACS assays (Figs. 2 and 5). Thus, our results clearly indicate that not only the HPC capable of differentiating into mature blood cells (week 0 HPC) are $HLA\text{-}DR^+$ and $HLA\text{-}DQ^-$, but also that the HPC responsible for the sustained proliferation of CFU-GM, BFU-E, and CFU-GEMM in vitro express HLA-DR determinants, and lack DQ antigens.

Although similar results have been preliminarily reported by some investigators (17), others (16, 18) have reported that the capacity of bone marrow cells to produce HPC in long-term culture systems after treatment of the (re)inoculum with anti-HLA-DR and complement was not significantly reduced. Therefore, we investigated whether precursor cells with no, or only low HLA-DR expression were capable of producing relatively more HPC after reinoculation than HLA-DR^+ precursor cells. As shown in Table II, there appeared to be no differences in proliferation potential between HPC with low HLA-DR expression and HPC with high HLA-DR expression.

Based on these findings and the conflicting results of other investigators (4, 5, 19), we hypothesize that, although (almost) all HPC capable of in vitro proliferation and differentiation appear to be HLA-DR^+, the degree of expression may differ considerably. The percentages of HLA-DR^+ HPC found may therefore depend on the sensitivity of the assay used, and may be influenced by differences in experimental conditions, such as differences in anti–class II antibodies, and complement sources or concentrations (19).

We hypothesize further that, since it has been shown (5, 21) that at least part of the regulation of hematopoiesis is strongly associated with HLA-DR expression, the degree of expression of class II molecules on HPC may be an important factor in the regulation of hematopoiesis. The more HLA-DR molecules are expressed on HPC, the more these HPC may be subject to regulatory humoral and cellular agents (21).

Our results open the possibility of studying class II–associated regulation of HPC proliferation in liquid long-term cultures. Furthermore, because HLA-DQ antigens are not expressed on HPC, HLA-DQ^+ neoplastic cells (e.g. mature B cell lymphomas; 22) can be removed from autologous bone marrow grafts using anti-HLA-DQ antibodies. Alternatively, if the human true pluripotential stem cells express HLA-DR antigens, positive selection of stem cells for bone marrow transplantation may be achieved using anti-HLA-DR antibodies.

Summary

The expression of HLA-DR antigenic determinants on human hematopoietic progenitor cells (HPC) capable of differentiating into mature blood cells, as determined in semisolid cultures, has been demonstrated previously (3–7). Here, we investigated the expression of class II determinants on HPC responsible for the sustained proliferation of colony-forming units of granulocyte/macrophage (CFU-GM), of multilineage HPC (CFU-GEMM, granulocyte/erythrocyte/macrophage/megakaryocyte), and burst-forming units of erythroid cells (BFU-E) in liquid long-term cultures. Using both fluorescence-activated cell sorting and complement-dependent cytotoxicity assays, HLA-DR determinants could be identified on virtually all these HPC capable of proliferating in long-term cultures. Experiments in which the stromal layer had been irradiated provided evidence that the HPC themselves were truly HLA-DR^+, and that the sustained proliferation of HPC was not due to activation of HLA-DR^- residual HPC in the stromal layer by reinoculated HLA-DR^+ accessory cells. Furthermore, it was shown that all HPC recognized in semisolid and liquid long-term cultures were HLA-DQ^-. These results suggest that the human true pluripotential stem cell is

HLA-DR$^+$. These results open the possibility of studying class II–dependent regulation of hematopoiesis in liquid long-term cultures.

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