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Qualitative and Quantitative Heterogeneity in Pgp-1 Expression among Murine Thymocytes[1]

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A proportion of Pgp-1$^+$ cells in the thymus have been shown to have progenitor activity. In adult AKR/Cum mice the total Pgp-1$^+$ population in the thymus differs from that of the bulk of thymocytes and is antigenically heterogeneous when examined by flow cytometry. Pgp-1$^+$ thymocytes are enriched for several minor cell populations compared to total thymocytes: B2A2$^-$, interleukin-2-receptor$^+$ (IL-2R$^+$), and Lyt-2$^-$, L3T4$^-$. However, these subsets are still a minor proportion of the Pgp-1$^+$ cells, the majority being Lyt-2$^+$ and/or L3T4$^+$ and B2A2$^+$. Pgp-1$^+$ thymocytes also differ from the bulk of thymocytes in having lower amounts of Thy-1 and in showing a higher proportion of single positive (Lyt-2$^+$, L3T4$^-$ or Lyt-2$^-$, L3T4$^+$) cells. Populations of adult thymocytes that are enriched in progenitor cells can be isolated by cytotoxic depletion using either anti-Thy-1 antibody (Thy-1 depletion) or anti-Lyt-2 and anti-L3T4 antibody (Lyt-2, L3T4 depletion). Pgp-1$^+$ cells in progenitor cell-enriched populations are also phenotypically heterogeneous. Pgp-1$^+$ cells in both populations may be IL-2R$^+$ or IL-2R$^-$ and B2A2$^+$ or B2A2$^-$. The population of Pgp-1$^+$ cells in progenitor cell-enriched populations in the adult differs from that of the fetus at 14 days of gestation in that in the 14-day fetus, most Pgp-1$^+$ cells are IL-2R$^+$. By Day 15 of gestation, distinct populations of Pgp-1$^+$, IL-2R$^-$; Pgp-1$^+$, IL-2R$^+$; and Pgp-1$^-$, IL-2R$^+$ cells are observed. In the 15-day fetus, as in the adult, many Pgp-1$^+$ thymocytes express low to moderate levels of Thy-1. The total percentage of Pgp-1$^+$ cells in the thymus varies among different mouse strains, ranging from 4 to 35% in the thymus of young adult mice. Pgp 1.1 strains contain more detectably Pgp-1$^+$ thymocytes than Pgp 1.2 strains; however, there is variability in the proportion of Pgp-1$^+$ cells, even among Pgp 1.2 strains. In contrast to AKR/Cum mice, the Pgp-1$^+$ thymocyte population in BALB/c mice, which contain a high proportion of Pgp-1$^+$ thymocytes, closely resembles the total thymocyte population.

INTRODUCTION

The Pgp-1 glycoprotein is expressed on a subset of mouse thymocytes (1). In adult AKR/Cum and C57BL/6 mice, only 5–10% of thymocytes are strongly Pgp-1$^+$ by flow cytometry (2–4), although an additional, variable number of weakly Pgp-1$^+$ cells that overlaps the background population is often seen (5). We have demonstrated that the intrathymic thymus-homing progenitor that homes to and transiently colonizes the thymus upon intravenous injection is contained within the Pgp-1$^+$ population of thymocytes (5, 6). Populations of adult thymocytes that are enriched in intrathymic thymus-homing progenitors, either by cytotoxic depletion with anti-Thy-1 antibody and complement (5, 6) or by cytotoxic depletion with anti-Lyt-2 and anti-

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L3T4 antibody and complement (7), are also enriched in Pgp-1$^+$ cells (3–6). The intrathymic thymus-homing progenitor, however, is likely to represent only a minor proportion of the Pgp-1$^+$ cells within the thymus (6), and the phenotypic heterogeneity of the total population of Pgp-1$^+$ thymocytes is not well defined. We have demonstrated that most Pgp-1$^+$ cells in progenitor cell-enriched populations in the adult lack detectable IL-2R,[2] although a minority population of Pgp-1$^+$, IL-2R$^+$ cells does exist (6).

In this paper we address three points regarding the phenotypic heterogeneity of the population of Pgp-1$^+$ cells in the thymus. First, we examine the expression of other cell surface markers both on the total unselected Pgp-1$^+$ cell population of the adult AKR/Cum mouse thymus and on the progenitor cell-enriched populations isolated from adult thymocytes of AKR/Cum mice by cytotoxic depletion either with anti-Thy-1 antibody and complement (5, 6) or with anti-L3T4 and anti-Lyt-2 antibody and complement (7). Second, we examine the extent to which the distribution of Pgp-1$^+$, IL2R$^-$; Pgp-1$^+$, IL-2R$^+$; and Pgp-1$^-$, IL2R$^+$ cells in the fetal thymus at various stages of ontogeny differs from that of the progenitor cell-enriched populations in the adult. Finally, we note that the proportion of cells in the adult thymus that are Pgp-1$^+$ may vary considerably, depending on the mouse strain examined, and we compare the phenotype of the total Pgp-1$^+$ population in the thymus of BALB/c mice, which express a high proportion of Pgp-1$^+$ cells, with that of AKR/Cum mice, which contain a low proportion of Pgp-1$^+$ cells in the thymus.

MATERIALS AND METHODS

Flow cytometry. Analysis of cell surface antigen expression by flow cytometry was carried out on a modified Los Alamos flow microfluorimeter as described (5, 6). Propidium iodide (5 μg/ml final concentration) was added to all samples to stain dead cells which were gated out during analysis.

Pgp-1 antigen expression was assayed using biotin-conjugated monoclonal antibody IM7.8.1 (1), followed by incubation with B-phycoerythrin-conjugated streptavidin, except in experiments involving fetal mice where biotin-conjugated antibody was followed by avidin and then biotin-conjugated B-phycoerythrin (6). IL-2R was assayed using monoclonal antibody PC61.5 (8). When Pgp-1 and IL-2R were assayed together, fluorescein-conjugated PC61.5 was used.

The B2A2 determinant (9, 10) was detected using rat monoclonal antibody R7 129.7 produced in this laboratory. R7 129.7 was obtained from a fusion of spleen cells from a rat immunized with the erythroleukemia cell line 745.6 and the murine myeloma Sl94/5.XXO.BU.1 and was shown to detect the B2A2 determinant by a competition assay. When B2A2 and Pgp-1 or IL-2R were assayed together, fluorescein-conjugated R7 129.7 antibody was used in conjunction with biotin-conjugated IM7.8.1 or PC61.5, followed by B-phycoerythrin-conjugated streptavidin.

Lyt-2 was assayed using either the monoclonal antibody 53.6.72 (11) or the IgM monoclonal antibody 3.155.2 (12), while L3T4 was assayed using either the monoclonal antibody GK 1.5 (13) or the IgM monoclonal antibody RL 172.4 (8). When L3T4 and Lyt-2 were assayed together, biotin-conjugated GK 1.5 was used followed by B-phycoerythrin-conjugated streptavidin and fluorescein-conjugated 53.6.72. When Pgp-1 was assayed together with Lyt-2 and/or L3T4, L3T4 was detected using

[2] Abbreviations used: IL-2R, interleukin-2 receptor; FALS, forward angle light scatter.

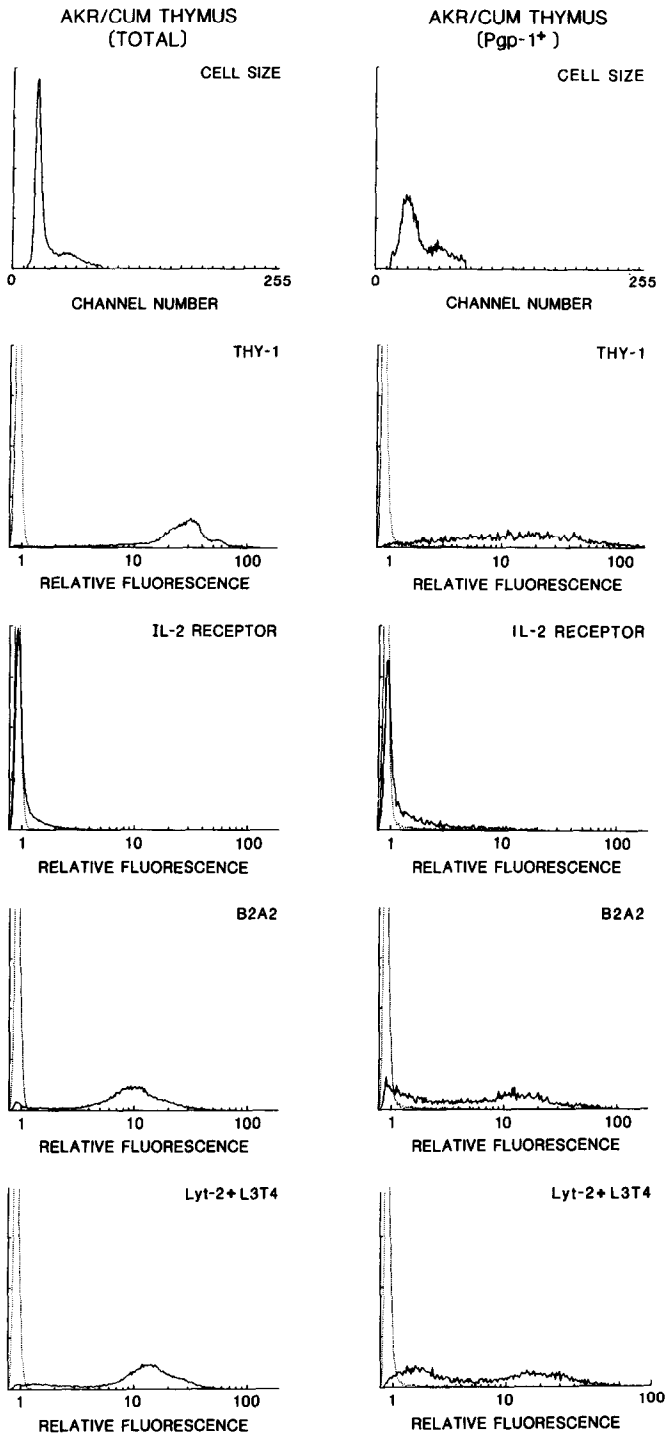

FIG. 1. Fluorescence profiles of total AKR/Cum thymocytes and of the Pgp-1$^+$ cells within this population. Thymocytes from pooled 5-week-old animals were stained with biotin-conjugated *anti*-Pgp-1 antibody followed by B-phycoerythrin-conjugated streptavidin and either fluorescein-conjugated anti-Thy-1, fluorescein-conjugated anti-B2A2, or IgM anti-Lyt-2 and IgM anti-L3T4 followed by fluorescein-conju-

RL 172.4, followed by fluorescein-conjugated goat anti rat μ-chain-specific antibody, while Lyt-2 was detected either using fluorescein-conjugated 53.6.72 or using 3.155.2 followed by fluorescein-conjugated anti-rat μ-chain-specific antibody.

Thy-1 was detected using fluorescein-conjugated monoclonal antibody T24/31.7 (14).

Isolation of thymocyte subpopulations. All subpopulations were isolated from the thymocytes of 4- to 5-week-old AKR/Cum mice.

Thy-1 depletion was carried out as described in (5, 6). Briefly, thymocytes were incubated in tissue culture supernatant containing the anti-Thy-1.2 monoclonal antibody HO 13.4.9 (final dilution 1:4) and rabbit complement (final dilution 1:10) at 37°C for 45 min. After washing, viable thymocytes were isolated on a Ficoll–Hypaque gradient.

Lyt-2, L3T4 depletion was carried out by treating pools of 1×10^9 thymocytes in a final volume of 10 ml with tissue culture supernatants of the anti-Lyt-2 monoclonal antibody 3.155.2 (final dilution 1:5) and the anti-L3T4 monoclonal antibody GK 1.5 (final dilution 1:5) and rabbit complement (final dilution 1:10) at 37°C for 45 min. After washing and the isolation of viable thymocytes on a Ficoll–Hypaque gradient, the remaining cells were suspended at 1×10^7/ml in phosphate-buffered saline containing 2.5% newborn calf serum. These cells (3×10^7/plate) were added to 100-mm petri dishes to which DEAE-purified rabbit anti-rat Ig had been bound and incubated at 0°C for 90 min. The nonattached cells were recovered by gently washing the plates three times with cold phosphate-buffered saline containing 2.5% newborn calf serum.

B2A2 depletion was carried out similarly to Lyt-2, L3T4 depletion with R7 129.7 tissue culture supernatant (final dilution 1:5) substituted for 3.155.2 and GK 1.5.

Fetal thymocytes. Thymocytes were obtained from fetal AKR/J or AKR/Cum mice as described in (2). The occurrence of a vaginal plug was considered as Day 0 of gestation.

RESULTS

The phenotype of Pgp-1$^+$ thymocytes and the phenotype of total thymocytes compared. The intrathymic thymus-homing progenitor has been shown to be Pgp-1$^+$ (5, 6). To assess the heterogeneity among the Pgp-1$^+$ cells of the thymus we stained total AKR/Cum thymocytes simultaneously for Pgp-1 and for a variety of other cell surface markers. The staining of the total thymus population and of the Pgp-1$^+$ cells within it is shown in Fig. 1. Using forward angle light scatter (FALS) as a measure of cell size (15), the Pgp-1$^+$ population is represented selectively among intermediate–large cells from AKR/Cum mice (Fig. 1, cell size). While 81% of total thymocytes fall below an arbitrary FALS threshold dividing small and large thymocytes, only 66% of

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gated goat anti μ-chain-specific antibody. List mode files of 50,000 cells were collected. To obtain the three-decade fluorescence histogram of the total thymocyte population, list mode files were gated to eliminate dead cells and cells falling outside the size distribution of single cells. To obtain the fluorescence of the Pgp-1$^+$ cells, the same list mode files were additionally gated to include only those cells in the phycoerythrin channel above an arbitrary threshold such that 2–3% of cells were scored as positive when B-phycoerythrin-conjugated streptavidin alone was used. In this experiment 9% of total gated cells were included as Pgp-1$^+$. Dotted curves represent background fluorescence with buffer substituted for directly fluoresceinated reagents or unconjugated IgM antibody. Solid curves represent specific fluorescence. Integrated forward angle light scatter is used as a measure of cell size. Ordinate: cell number.

Pgp-1$^+$ thymocytes fall below this threshold. The mean FALS signal of the Pgp-1$^+$ thymocytes falling below this threshold is approximately 1.2 times that of the total thymocytes falling below this threshold. Of the thymocytes above this threshold, the mean FALS signal of the Pgp-1$^+$ cells is only slightly larger than that of the total population (ratio of 1.05).

The Pgp-1$^+$ population shows a heterogeneous distribution of Thy-1 fluorescence with a mean fluorescence considerably lower than that of the total population (Fig. 1, Thy-1). While only 6% of total thymocytes are B2A2$^-$, 26% of Pgp-1$^+$ thymocytes are B2A2$^-$ (Fig. 1, B2A2). The mean B2A2 fluorescence of the B2A2$^+$, Pgp-1$^+$ cells is, however, slightly greater than the mean fluorescence of the population of B2A2$^+$ cells in total thymus, indicating that the Pgp-1$^+$ population is selectively enriched for the most highly fluorescent of the B2A2$^+$ cells as well as for the least fluorescent. It is unlikely that non-T cells contribute significantly to the Pgp-1$^+$ subset as only 4% of Pgp-1$^+$ cells are B220$^+$ (not shown).

The Pgp-1$^+$ population is enriched for several minor subsets that predominate in progenitor cell-enriched populations (see below). A higher proportion of Pgp-1$^+$ cells are highly fluorescent for IL-2R (7%) compared to the total thymocyte population (1%) (Fig. 1, IL-2R). The proportion of Pgp-1$^+$ cells that are Lyt-2$^-$, L3T4$^-$ (20%) is also higher than that in the total population (6%) (Fig. 1, Lyt-2 + L3T4).

Several lines of evidence (data not shown) suggest that cells with a phenotype resembling that of medullary cells (9, 16, 17) may be selectively represented among the Pgp-1$^+$ cells. The Pgp-1$^+$ population is enriched in cells staining for Lyt-2 or L3T4 alone. For example, although approximately 80% of Pgp-1$^+$ cells stain for Lyt-2 + L3T4, only approximately 60% of Pgp-1$^+$ cells stain for Lyt-2 alone, suggesting that about 20% of the Pgp-1$^+$ thymocytes must be Lyt-2$^-$, L3T4$^+$ compared to about 6% of total thymocytes. The Pgp-1$^+$ cells also show an increased number of cells staining strongly for Ly-1 (11% of total thymocytes vs 35% of Pgp-1$^+$ thymocytes) and a decreased number of cells staining for ThB (69% of total thymocytes vs 45% of Pgp-1$^+$ thymocytes). The increased number of intermediate-sized and of Thy-1 intermediate-low cells in Pgp-1$^+$ thymocytes compared to total thymocytes is also consistent with the idea that some Pgp-1$^+$ cells have a phenotype resembling that of medullary thymocytes. Some Pgp-1$^+$ cells, however, do clearly resemble cortical-type cells.

Pgp-1$^+$ cells in Thy-1-depleted, in Lyt-2, L3T4-depleted, and in B2A2-depleted thymocyte populations. Populations of adult thymocytes enriched in intrathymic thymus-homing progenitors have been obtained either by cytotoxic depletion with anti-Thy-1 antibody and complement (Thy-1 depletion (5, 6)) or by cytotoxic depletion with anti-Lyt-2 and anti-L3T4 antibodies and complement (Lyt-2, L3T4 depletion (7)). The former procedure enriches for a population of mostly Thy-1$^+$ cells, showing a heterogeneous distribution of Thy-1 fluorescence which for unknown reasons is resistant to the cytotoxic action of anti-Thy-1 antibody and complement treatment (5, 6, 18). This Thy-1-depleted population is substantially enriched for Pgp-1$^+$ cells (5, 6, 18), as is the Lyt-2, L3T4-depleted cell population (3, 4). In addition to Pgp-1$^+$ cells, both of these populations have also been shown to be enriched for IL-2R$^+$ cells (3, 6, 8, 19), and the Lyt-2$^-$, L3T4$^-$ population has been reported to contain B2A2$^-$ cells (10), some or all of which have been considered to be early thymocytes (9, 20), and many of which are Pgp-1$^+$ (3, 20).

To study the phenotype of the Pgp-1$^+$ cells in thymocyte subpopulations enriched for "early" thymocytes, we prepared Thy-1-depleted, Lyt-2, L3T4-depleted, and

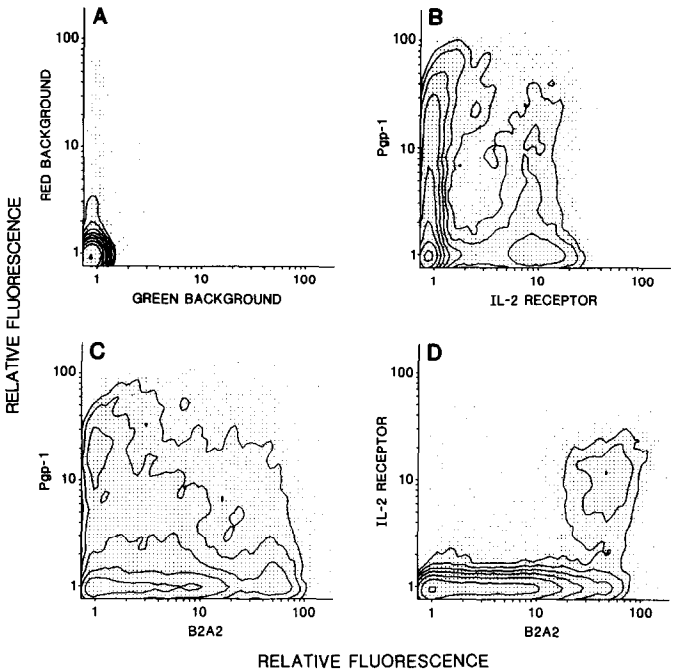

FIG. 2. Two-color immunofluorescence analysis of a Thy-1-depleted subpopulation isolated from AKR/Cum mice. This subpopulation was isolated as described under Materials and Methods from the same pool of thymocytes as that used to isolate the population of Lyt-2, L3T4-depleted thymocytes illustrated in Fig. 3. The subpopulation is characterized in Table 1. Dots indicate two or more events (raw data). Contours were drawn following application of a bivariate curve-smoothing routine and outline successive doubling numbers of events starting at two events (smoothed data).

B2A2-depleted thymocytes from AKR/Cum mice (Table 1) and studied the phenotype of the cells by two-color immunofluorescence (Figs. 2, 3).

As we have shown previously (5, 6, 18), nearly all of the cells surviving cytotoxic treatment with anti-Thy-1 and complement are still Thy-1$^+$; however, this population differs from the total thymocyte population in being substantially enriched in Pgp-1$^+$ and IL-2R$^+$ cells (Table 1, Fig. 2B). As we have previously described (6), most of these Pgp-1$^+$ and IL-2R$^+$ cells bear only one of these two markers, although a proportion of cells stain for both Pgp-1 and IL-2R (28% of the total of the cells staining with one or the other antibody in this experiment). About 25% of the cells in the Thy-1-depleted population are B2A2$^-$, a substantial enrichment over total thymocytes. The Pgp-1$^+$ cells are found in both the B2A2$^+$ and the B2A2$^-$ populations (Fig. 2C). Over 85% of the Pgp-1$^+$ cells are B2A2$^+$, although some of these Pgp-1$^+$, B2A2$^+$ cells stain very weakly for B2A2. Although most B2A2$^-$ cells are Pgp-1$^+$, there does appear to be a small population of cells that are B2A2$^-$ and Pgp-1$^-$. The IL-2R$^+$ cells are nearly all B2A2$^+$ (94% of IL-2R$^+$ cells are B2A2$^+$ in this experiment) and are concentrated among the brightest of the B2A2$^+$ cells (Fig. 2D). Substantial numbers of Lyt-2$^+$ and L3T4$^+$ cells are present in the Thy-1-depleted population (Table 1): 46% of the Lyt-2$^+$ cells and 45% of the L3T4$^+$ cells were Pgp-1$^+$ in this experiment (data not shown).

The Lyt-2, L3T4-depleted population isolated by us appears comparable to those isolated by others (3, 8, 20) in containing about 25% Pgp-1$^+$ and 50–60% IL-2R$^+$ cells

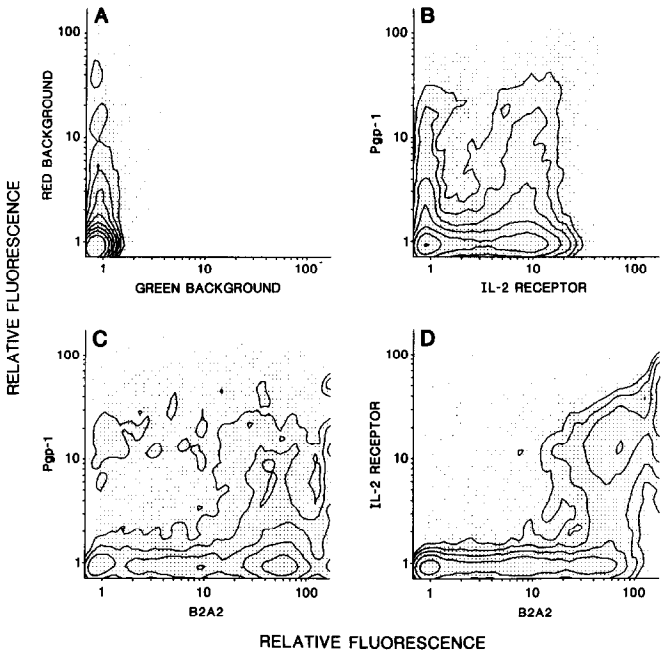

FIG. 3. Two-color immunofluorescence analysis of an Lyt-2, L3T4-depleted subpopulation isolated from AKR/Cum mice. This subpopulation was isolated as described under Materials and Methods from the same pool of thymocytes as that used to isolate the population of Thy-1-depleted thymocytes illustrated in Fig. 2. The subpopulation is characterized in Table 1. Dots indicate two or more events (raw data). Contours were drawn as in Fig. 2.

(Table 1). We consistently observe 15–30% L3T4$^+$ cells in our Lyt-2, L3T4-depleted populations (Table 1). These L3T4 cells are very weakly fluorescent with a mean fluorescence 8- to 10-fold below the L3T4 fluorescence of the total thymocyte population, comparable to the L3T4 fluorescence seen in typical "double-negative" populations (7); however, they do score as positive under our analytical conditions when their fluorescence is compared to that seen when the buffer is substituted for specific antibody. (The Pgp-1$^+$ cells in the Lyt-2, L3T4-depleted population cannot be solely accounted for by the small number of L3T4 cells remaining in this population as only about half of these L3T4$^+$ cells are detectably Pgp-1 (data not shown). If these cells are eliminated from consideration, then about 20% of the total L3T4$^-$, Lyt-2$^-$ cells are Pgp-1$^+$.)

As is the case with the Thy-1-depleted population, most of the cells in the Lyt-2, L3T4-depleted population staining for Pgp-1 or IL-2R stain for only one of these two markers (Fig. 3B), although a subset of cells (about one-third of the total of the cells staining with one or the other antibody in this experiment) are Pgp-1$^+$, IL-2R$^+$. Nearly all (>90%) of the Pgp-1$^+$ cells in the Lyt-2, L3T4-depleted population are B2A2$^+$ (Fig. 3C), although small subsets of Pgp-1$^+$, B2A2$^-$ cells and Pgp-1$^-$, B2A2$^-$ cells are also present. Essentially all IL-2R$^+$ cells in this population are B2A2$^+$ (Fig. 3D).

In order to examine the phenotype of the B2A2$^-$ cells more exactly, we used antibody and complement treatment followed by panning to isolate a B2A2$^-$ population from AKR/Cum mice (Table 1). This population represents 0.7% of the total thymo-

TABLE 1

Phenotype of Thymocyte Populations Enriched in Pgp-1$^+$ Cells[a]

Antigen	% Positive cells in population			
	Untreated AKR/Cum thymus	Thy-1 depleted	Lyt-2, L3T4 depleted	B2A2 depleted
Thy-1	96	96	96	93
Lyt-2	90	29	2	37
L3T4	95	53	17	29
B2A2	95	75	87	7[b]
Pgp-1	7	46	28	81
IL-2R	2	26	60	5

[a] Isolated from AKR/Cum thymocytes as described under Materials and Methods. Recoveries were Thy-1 depletion, 3.9%; Lyt-2, L3T4 depletion, 0.8%; B2A2 depletion, 0.7% of the initial cell input.

[b] Determined by reanalysis with R7129.7 tissue culture supernatant and fluorescein-conjugated anti-immunoglobulin. When reanalysis was done with biotin-conjugated R7129.7 followed by fluorescein-conjugated avidin, 49% of cells scored above background.

cytes. In agreement with the data of Scollay and Shortman using CBA/CaH WEHI mice (10), about 60% of these B2A2$^-$ cells are L3T4$^+$ or Lyt-2$^+$ and, of the positive cells, nearly all stain for only one or the other of the two markers (not shown). Over 80% of B2A2$^-$ cells are Pgp-1$^+$ and essentially none are IL-2R$^+$, in accordance with the observations of others on a subset of B2A2$^-$ cells isolated from Lyt-2, L3T4-depleted cell populations (3, 20).

Ontogeny of Pgp-1 and IL-2R expression. In adult AKR/Cum mice (6, this paper), Pgp-1$^+$, IL-2R$^+$ cells in progenitor cell-enriched populations represent a minority subset of the total cells staining for Pgp-1 and IL-2R. We have shown, however, that in 13- and 14-day fetal mice, the bulk of cells in the thymus are Pgp-1$^+$ (2), while others (8, 21, 22) have reported that many thymocytes are IL-2R$^+$ at 14–15 days of gestation. These two observations raise the question as to when during gestation the populations of Pgp-1$^+$, IL-2R$^-$ and Pgp-1$^-$, IL-2R$^+$ cells become predominant in the thymus. Data from two-color immunofluorescence analysis of the thymocytes of fetal mice from 14–17 days of gestation for Pgp-1 and IL-2R are shown in Figs. 4 and 5. At Day 14 of gestation most thymocytes are Pgp-1$^+$, IL-2R$^+$. At this time, staining for both Pgp-1 and IL-2R is quite heterogeneous and most IL-2R$^+$ cells are only weakly fluorescent (Fig. 5). By Day 15, the proportion of Pgp-1$^+$ cells has fallen considerably while the proportion of IL-2R$^+$ cells remains high. Distinct Pgp-1$^+$, IL-2R$^-$ and Pgp-1$^-$, IL-2R$^+$ populations are seen at this time, and many IL-2R$^+$ cells are highly fluorescent (Fig. 5). At 15 days of gestation only 23% of the total of cells staining for either Pgp-1 or IL-2R are Pgp-1$^+$, IL-2R$^+$, while 86% of the total of cells staining for either Pgp-1 or IL-2R stain for both markers at Day 14. Over the next 2 days the percentage of Pgp-1$^+$ cells in the thymus continues to decline while the percentage of IL-2R$^+$ cells declines after Day 16. The proportion of Pgp-1$^+$, IL-2R$^+$ cells also declines as a fraction of the total cells in the thymus. Throughout the period from Day 15 to Day 17, however, about half of all Pgp-1$^+$ cells are also IL-2R$^+$. When the Pgp-1$^+$ and IL-2R$^+$ cells in the thymus at Day 15 are examined for Thy-1, a further

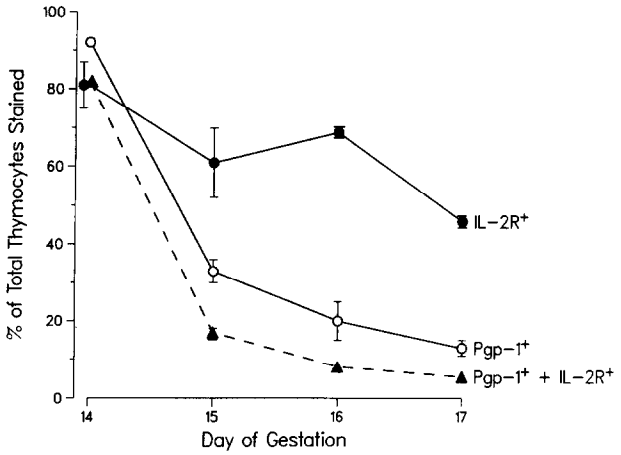

FIG. 4. Percentage of Pgp-1$^+$, IL-2R$^+$ and Pgp-1$^+$, IL-2R$^+$ thymocytes in fetal AKR/J and AKR/Cum mice of the indicated gestational age. Two-color analytical flow cytometry was carried out as described under Materials and Methods. Error bars indicate the range of two independent measurements of positive cells in the same experiment, except at 15 days of gestation where measurements from three independent experiments were averaged and the range among the experiments is indicated by the error bars.

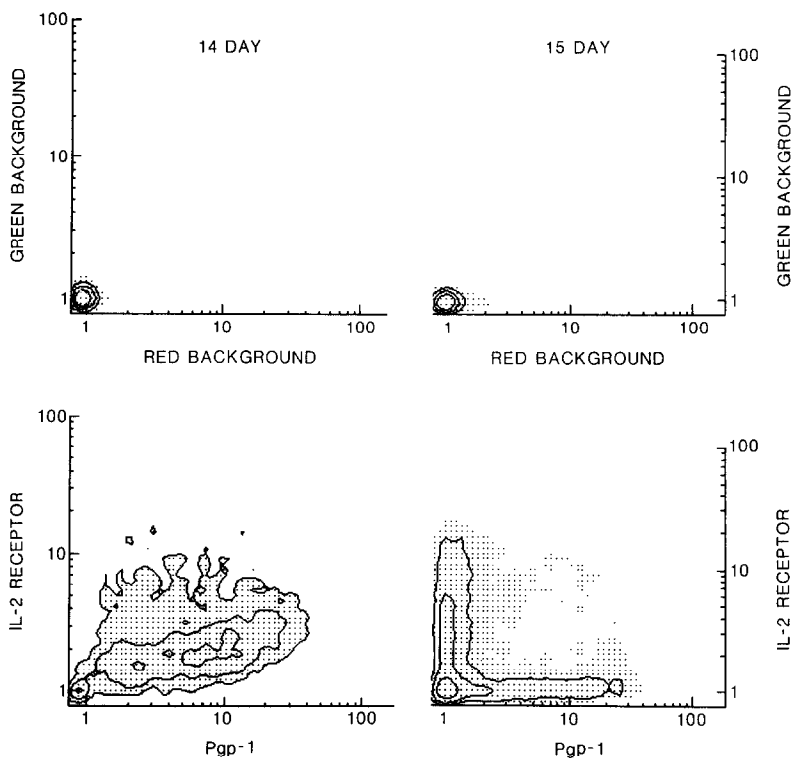

FIG. 5. Two-color immunofluorescence analysis for Pgp-1 and for IL-2R on thymocytes from fetal AKR/Cum mice of 14 and 15 days gestation. Analytical flow cytometry was carried out as described under Materials and Methods, with biotinylated antibody followed by avidin- and then biotin-conjugated B-phycoerythrin. Data was mapped to include >95% of total gated events; isometric contours represent 25, 50, and 75% of total gated events.

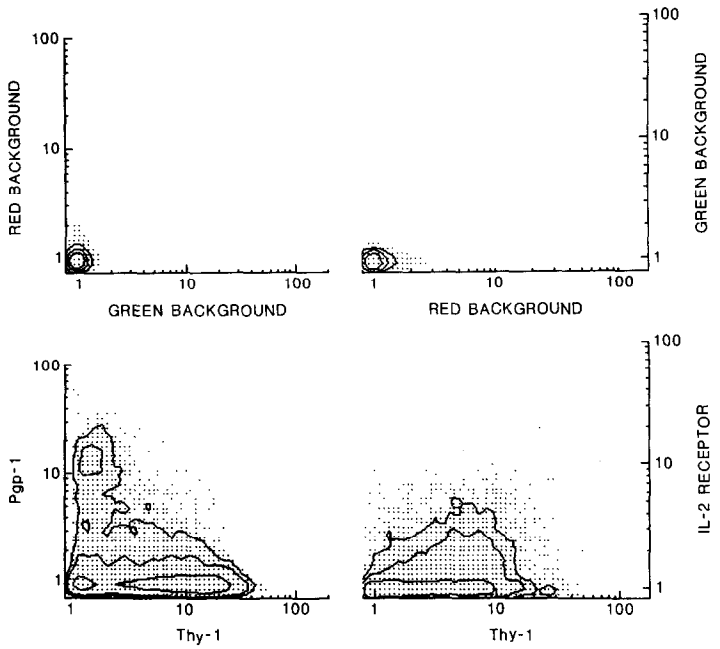

FIG. 6. Two-color immunofluorescence analysis for Thy-1 and for Pgp-1 or IL-2R on thymocytes from fetal AKR/Cum mice of 15 days gestation. Fluorescein-conjugated anti-Thy-1 antibody (T24/31.7) was used with biotin-conjugated anti-Pgp-1 antibody (IM7.8.1), avidin- and biotin-conjugated B-phycoerythrin. Biotin-conjugated anti-Thy-1 antibody and avidin- and biotin-conjugated B-phycoerythrin were used with fluorescein-conjugated anti-IL-2R antibody (PC61.5). Data were mapped to include >95% of total gated events; isometric contours represent 25, 50, and 75% of total gated events.

difference becomes apparent (Fig. 6). Many of the cells in the thymus that express the most Pgp-1 express little or no Thy-1, while those cells that express the most IL-2R also express the most Thy-1.

Expression of Pgp-1 antigen on the thymocytes of various mouse strains. The Pgp-1 antigen is expressed on only 5–10% of the thymocytes of AKR/Cum and C57BL/6 mice (6, 18, 20). We note, however, that the percentage of Pgp-1^+ cells as a percentage of total thymocytes shows considerable variation among mouse strains and may be as high as 25–35% (Table 2). It is of interest that the three Pgp 1.1 strains examined consistently show the highest percentage of Pgp-1^+ cells in the thymus. F_1 hybrid mice between Pgp 1.1 and Pgp 1.2 strains coexpress the Pgp 1.1 and Pgp 1.2 determinants (23). The percentage of Pgp-1^+ cells in the thymus of F_1 hybrids between Pgp 1.1 and Pgp 1.2 strains is always higher than that seen in the respective Pgp 1.2 parent (Table 2, and data not shown). The fact that Pgp 1.1 strains show a higher percentage of Pgp-1^+ thymocytes is not due to a greater affinity of the IM7.8.1 monoclonal antibody for the Pgp 1.1 determinant, since the same results were seen when a panel of anti-Pgp-1 monoclonal antibodies was used.

When the population of Pgp-1^+ cells in the thymus of BALB/c mice is compared to the population of total thymocytes (Fig. 7), it is seen that the population of Pgp-1^+ cells in BALB/c mice is much more similar to the total thymocyte population than that of AKR/Cum mice (compare Figs. 1 and 7). Based on the criterion of forward angle light scatter, the distribution of small and large Pgp-1^+ cells in BALB/c mice is nearly identical to that in the total thymocyte population, in contrast to AKR/Cum

TABLE 2

Percentage of Pgp-1$^+$ Cells in the Thymus of Mice of Different Strains

Strain	Pgp-1 allele[a]	%Pgp-1$^+$ thymocytes[b]	
		Experiment 1	Experiment 2
C3H/HeJ	2	4	5
AKR/J	2	6	4
C57BL/6J	2	6	4
SJL/J	2	13	—
A/J	2	15	8
DBA/2J	1	26	12
CBA/J	1	28	—
BALB/c	1	37	24
(BALB/c × C57BL/6)F_1	1/2	23	—
(BALB/c × A/J)F_1	1/2	—	12
(C3H/HeJ × DBA/2J)F_1	1/2	—	9

[a] Based on data in Ref. (23).

[b] Determined by staining thymocytes of 4- to 8-week-old mice with tissue culture supernatants containing monoclonal antibody IM7.8.1, recognizing a nonpolymorphic determinant of Pgp-1 (1), followed by fluorescein-conjugated goat anti-rat immunoglobulin. Positive cells are considered to be cells above an arbitrary threshold such that background staining was 2–5% in control samples, in which normal tissue culture medium was substituted for specific monoclonal antibody. At these settings many "positive" cells are only weakly fluorescent. % Positive cells = ((% cells stained above threshold with specific antibody) − (% cells stained above threshold with normal medium)).

mice, where the Pgp-1$^+$ cells were enriched in intermediate–large cells. Furthermore, in the BALB/c thymus the fluorescence distribution of Pgp-1$^+$ cells more closely resembles that of the total thymocyte population when both populations are examined for their expression of Thy-1, B2A2, IL-2R, and Lyt-2 + L3T4. There is, however, a slight enrichment for intermediate–low Thy-1, B2A2$^-$, and Lyt-2$^-$, L3T4$^-$ cells among the Pgp-1$^+$ thymocytes of BALB/c mice.

DISCUSSION

The subset of cells in the thymus that expresses the Pgp-1 glycoprotein is of interest since the intrathymic thymus-homing progenitor, a minority adult thymocyte population that is able to home to the thymus after intravenous injection and to transiently repopulate this organ, has been demonstrated to be Pgp-1$^+$ (5, 6). This paper has addressed the question of the heterogeneity of the Pgp-1$^+$ cells within the total thymocyte population and within subpopulations of thymocytes that have been shown to be enriched in thymus-homing progenitors. We have shown that the Pgp-1$^+$ population within the adult thymus is quite heterogeneous, that the phenotype of the predominant Pgp-1$^+$ population within the thymus changes during ontogeny, and that the proportion of Pgp-1$^+$ cells within the adult thymus can show qualitative and quantitative differences, depending on the mouse strain examined.

In AKR/Cum mice Pgp-1$^+$ thymocytes show clear differences from the total thymocyte population (Fig. 1). The Pgp-1$^+$ cells are enriched in cells of intermediate–large size and many Pgp-1$^+$ cells express low–intermediate levels of Thy-1, although nearly all Pgp-1$^+$ cells clearly are Thy-1$^+$. Most Pgp-1$^+$ cells (80%) in AKR/Cum mice

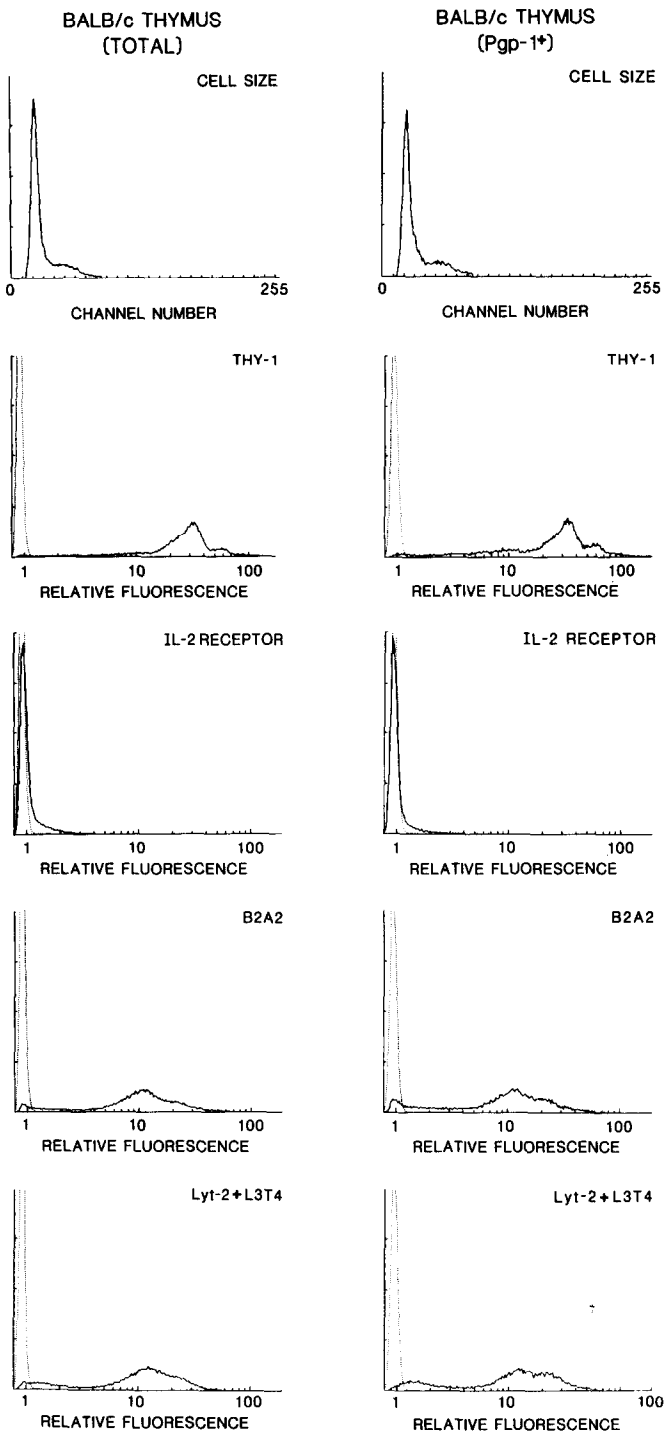

FIG. 7. Fluorescence profiles of total BALB/c thymocytes and of the Pgp-1^+ cells within this population. Thymocytes obtained from pooled 5-week-old animals were stained and analyzed as described in the legend to Fig. 1. In this experiment, 36% of total gated cells were included as Pgp-1^+. Dotted curves represent background fluorescence with buffer substituted for directly fluoresceinated reagents or unconjugated IgM antibody. Solid curves represent specific fluorescence. Integrated forward angle light scatter is used as a measure of cell size. Ordinate: cell number.

stain for L3T4 and Lyt-2, implying that most Pgp-1$^+$ cells are relatively "mature." The Pgp-1$^+$ thymocyte population is, however, enriched in Lyt-2$^-$, L3T4$^-$ cells and in cells bearing high levels of IL-2R.

Various indirect lines of evidence (an increased proportion of highly fluorescent Ly-1 cells, a decreased proportion of ThB$^+$ cells, an increase in the proportion of cells not staining with Lyt-2 or L3T4 when these markers are examined individually, as well as the cell size and Thy-1 distributions alluded to above) suggest that the Pgp-1$^+$ population in AKR/Cum mice is enriched in cells with a phenotype resembling that of medullary thymocytes. Budd *et al.* (20), however, did not observe any increase in Pgp-1$^+$ cells when the cortisone-resistant thymocytes of C57BL/6 mice were examined. This apparent difference may be a consequence of strain differences or due to the fact that the enrichment of those cells with an apparent medullary phenotype is too small to have been detectable when only the total number of Pgp-1$^+$ cells present is examined. Alternatively, some or all of the Pgp-1$^+$ cells with an apparent medullary phenotype may not, in fact, be typical of cells in the medulla when other characteristics, such as cortisone resistance, are examined.

Most Pgp-1$^+$ cells are B2A2$^+$; however, the Pgp-1$^+$ population is enriched in B2A2$^-$ cells. Many, although probably not all, B2A2$^-$ cells in the thymus are Pgp-1$^+$, since a B2A2$^-$ population isolated by cytotoxic ablation and panning was >80% Pgp-1$^+$. Over half of these B2A2$^-$ cells showed an Lyt-2$^+$, L3T4$^-$ or Lyt-2$^-$, L3T4$^+$ phenotype and must comprise a proportion of those Pgp-1$^+$ cells showing a "medullary-type" phenotype. There is evidence that B2A2$^-$, L3T4$^-$, Lyt-2$^+$ cells behave like peripheral T cells in functional assays (24), suggesting the possibility that some of the Pgp-1$^+$, B2A2$^-$ cells may be relatively mature. Further experiments, however, are needed to prove or disprove this idea. Most of the B2A2$^-$, Lyt-2$^-$, L3T4$^-$ cells must comprise a proportion of the small number of Lyt-2$^-$, L3T4$^-$, Pgp-1$^+$ cells seen in the total thymocyte population. Not all L3T4$^-$, Lyt-2$^-$, Pgp-1$^+$ cells are B2A2$^-$; however, as within the total Lyt-2, L3T4-depleted population, most Pgp-1$^+$ cells are also B2A2$^+$ (Fig. 3). The nature of the B2A2$^-$, L3T4$^-$, Lyt-2$^-$ population is uncertain, since no progenitor activity has been demonstrated within it (25, and our unpublished results) and it has been shown to contain cells expressing a low level of cell surface T-cell receptor (20, 25).

Two cytotoxic depletion methods have been used to isolate populations of adult thymocytes enriched in intrathymic thymus homing progenitors: treatment with anti-Thy-1 antibody and complement (5, 6) and treatment with anti-L3T4 and anti-Lyt-2 antibody and complement (7). The fact that the bulk of Pgp-1$^+$ cells in AKR/Cum mice express low–intermediate levels of Thy-1 indicates that the former method "works" because the Pgp-1$^+$ intrathymic thymus-homing progenitor is selectively spared during treatment with anti-Thy-1 antibody and complement. A substantial proportion of the cells spared by this methodology are Lyt-2$^+$ and/or L3T4$^+$. Further analysis of these cells (not shown) demonstrates that about half are Pgp-1$^+$. These cells are eliminated when Lyt-2, L3T4 depletion is used, accounting, at least in part, for the difference in the proportion of Pgp-1$^+$ cells obtained by the two methodologies (see Table 1). Some of the Lyt-2$^+$ and/or L3T4$^+$ cells in the Thy-1-killed population are likely to be relatively mature, since the percentage of cells showing T-cell receptor β-chain genes in the germline configuration (18) increases when these cells are removed from the Thy-1-depleted population (unpublished results).

On a per cell basis, the Thy-1-depleted and Lyt-2, L3T4-depleted populations are essentially equivalent in their thymus-repopulating activity when injected intrave-

nously (6). Since on the order of 10^6 cells must be injected intravenously and 10^5 cells intrathymically (26) to achieve repopulation, it is likely that progenitor cells capable of thymus repopulation are only a minor proportion, even of these "progenitor cell-enriched" populations.

In contrast to the total AKR/Cum thymocyte population, the subpopulations obtained after either Thy-1 depletion or Lyt-2, L3T4 depletion of AKR/Cum thymocytes are composed primarily of Pgp-1^+ and IL-$2R^+$ cells (Table 1, Figs. 2 and 3). In both populations the majority of cells are Pgp-1^+, IL-$2R^-$ or Pgp-1^-, IL-$2R^+$; however, in both cases, up to about one-third of the cells staining for either marker stain for both. The significance of the Pgp-1^+, IL-$2R^+$ cells in the adult thymus is uncertain. We have shown that essentially all intrathymic thymus homing-progenitor activity remains when IL-$2R^+$ cells (which includes the Pgp-1^+, IL-$2R^+$ population) are removed from Thy-1-depleted populations by panning (6). However, progenitor activity can be demonstrated among IL-$2R^+$ cells since sorted IL-$2R^+$ cells injected intrathymically repopulate the thymus (26). There is circumstantial evidence that the IL-$2R^+$ population contains more "differentiated" progenitors than the IL-$2R^-$ population (including Pgp-1^+, IL-$2R^-$ cells) since the IL-$2R^+$ cells give an earlier repopulation than has been shown for the Pgp-1^+ cells (5, 26). However, there is no direct evidence concerning the nature of the precursor–product relationships among Pgp-1^+, IL-$2R^-$; Pgp-1^+, IL-$2R^+$; and Pgp-1^-, IL-$2R^+$ populations.

When these three phenotypic classes of thymocytes were examined during fetal development, we found that Pgp-1^+, IL-$2R^+$ cells were the majority cell type in the thymus at Day 14, but by Day 15 Pgp-1^+ IL-$2R^-$ and Pgp-1^-, IL-$2R^+$ thymocytes predominated (Figs. 4 and 5). Clearly the phenotype of the bulk of thymocytes at Day 14 is different than that at later times. Again, however, it is not possible to conclude anything about the lineage relationships among these three phenotypic classes from these observations. In both the 15-day fetus and the adult mice, the Pgp-1^+ cells tended to express low to moderate levels of Thy-1 compared to the majority of Thy-1^+ cells in the population (Fig. 6). We noted previously (2) a tendency for the thymocytes from Day 16 fetal mice that were among the most fluorescent Pgp-1^+ cells to be among the least fluorescent Thy-1^+ cells. The IL-$2R^+$ cells in the 15-day fetus, in contrast, tend to be among the most fluorescent Thy-1^+ cells.

In addition to the heterogeneity in Pgp-1 expression among various subclasses of thymocytes, we observed a further heterogeneity in the percentage of detectably Pgp-1^+ cells seen in the thymus of different mouse strains (Table 2). While Pgp 1.1 strains appear to have a significantly higher percentage of Pgp-1^+ cells than Pgp 1.2 strains, there is heterogeneity even among Pgp-1.2 strains. The significance of the fact that the three strains examined that contain the highest percentage of Pgp-1^+ cells in the thymus are all Pgp 1.1 is uncertain. More extensive genetic experiments will be required to determine the number and nature of the genes that act to determine the expression of Pgp-1 in different strains.

In BALB/c mice, which contain a high percentage of Pgp-1^+ thymocytes, the overall size and surface phenotypes of the Pgp-1^+ cells much more closely resemble those of the total thymocyte population than is the case for AKR/Cum mice. This suggests that the increase in the proportion of Pgp-1^+ cells in the thymus of BALB/c mice does not represent the selective expansion of one or more Pgp-1^+ cell lineages but rather represents the expression of Pgp-1 in cells which do not normally express this antigen. This expression could be due to activation of Pgp-1 expression in lineages which do not normally express Pgp-1 or it could represent a change in the timing of

gene activation within lineages that normally express Pgp-1. This alteration could affect either the Pgp-1 gene or one or more genes controlling expression of the Pgp-1 gene product on the cell surface. Both alternatives may be true. Our results do, however, suggest the possibility that, at least in certain strains, some mature thymocytes and even thymus emigrants may be Pgp-1^+ (4). It is noteworthy that in man about 50% of the thymocytes of young children are Pgp-1^+ (27). It may be that man expresses one or more genes similar to those that act in high Pgp-1 strains such as BALB/c and that consequently Pgp-1 is expressed on a greater number of cells within the thymus than it is in mouse strains such as C57BL/6 or AKR.

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