

Developmental Changes and Functional Properties of Human Memory T Cell Subpopulations Defined by CD60 Expression

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The present study was undertaken to examine developmental changes of T cells expressing CD60 and their functional properties. Three-color immunofluorescence analysis revealed that the CD60 antigen was preferentially expressed on a proportion of memory (CD45RO⁺) CD4⁺ T cells, but less on memory CD8⁺ T cells, while this antigen is undetectable in naive (CD45RO⁻) T cells. A frequency of memory CD4⁺ T cells expressing CD60 in the peripheral blood was negligible in newborns and gradually increased with advancing age. CD60⁺ memory CD4⁺ T cells showed stronger proliferative responses to PPD and produced higher levels of IL-4 and IL-10 than CD60⁻ ones, whereas production of IL-2 and IFN- γ was similarly found in both cell subpopulations. In addition, it was shown that efficient helper activity for Ig production by B cells was predominated in CD60⁺ memory CD4⁺ T cells. These results suggest that CD60 may be primarily expressed on the functionally differentiated memory effector cells among circulating CD45RO⁺ CD4⁺ T cells. © 1998 Academic Press

INTRODUCTION

The CD60 antigen has recently been identified as the terminal 9-O-acetylated disialosyl group predominantly expressed on ganglioside G_{D3} (1). To date, this structure has mainly been reported as a tumor-associated antigen on melanoma cells (2). Although the natural ligand for the CD60 antigen remains elusive, it has been demonstrated that both anti-CD60 and anti-G_{D3} antibodies can exert costimulatory effects on activation of T cells, implying that the CD60 antigen may be an important surface component in the activation pathway in T cells (3–6). However, the surface expression of CD60 is usually seen only in a limited fraction of human T cells (7, 8). The increased percentages of CD60⁺ cells were detected in rheumatoid synovial T cells (9), in cutaneous T cells from psoriasis patients (10), and in leukemic T cells from patients with cuta-

neous T cell lymphoma (11). Previous studies have indicated that the CD60⁺ subset of CD4⁺ T cells provides more efficient helper function for B cell differentiation into Ig-secreting cells compared to CD60⁻ CD4⁺ T cells (12). Intriguingly, CD60⁺CD8⁺ T cells have been found to mediate helper activity for B cell differentiation (13). Moreover, some differences of cytokine production patterns have been observed between CD60⁺ and CD60⁻ subsets of CD4⁺ and CD8⁺ T cells (8, 13).

Human peripheral T cells are functionally heterogeneous with respect to maturation and cell differentiation stages following external antigenic stimuli. It is now widely accepted that differential expression of various isoforms of the CD45 surface molecule can discriminate between antigen-inexperienced or naive T cells and previously antigen-primed or memory T cells (14–16). Although prior functional studies on CD60⁺ T cells as above have been carried out for whole blood T cells, investigations using anti-CD60 or anti-G_{D3} antibodies have suggested that CD60 expression may be present on a proportion of memory (CD45RA⁻CD45RO⁺) populations of T cells, but, if any, on few of naive T cells (8, 17, 18). In this study, we used the three-color immunofluorescence analysis to examine developmental changes in CD60 expression on memory-like (CD45RO⁺) populations of CD4⁺ and CD8⁺ T cells. In addition, based on the ability to respond to the recall antigen and to produce some cytokines such as IL-2, IL-4, IL-10, and IFN- γ , we analyzed whether CD60 expression could define a specified population of memory-like T cells. The results suggest that the CD60 antigen may be largely expressed on the functionally differentiated memory T cells in the peripheral blood.

MATERIALS AND METHODS

Monoclonal Antibodies and Reagents

FITC-conjugated anti-CD60 (M-T41 clone) mAb was kindly provided by Dr. S. Harvey (PharMingen, San Diego, CA) (12). Peridium-chlorophyll, a protein (Per-

CP)-conjugated anti-CD4 (Leu-3a), and anti-CD8 (anti-Leu-2a) mAbs were purchased from Becton Dickinson Immunocytometry Systems (San Jose, CA). PE-conjugated anti-CD45RO (Leu-45RO) and anti-CD20 (Leu-16) mAbs were similarly obtained from Becton Dickinson Immunocytometry Systems. FITC-conjugated CD14 (OKM14) mAb was from Ortho Diagnostic Systems (Tokyo, Japan). PHA were obtained from Sigma Chemical Co. (St. Louis, MO), and PPD was from Japan BCG Co., Ltd. (Tokyo, Japan). PWM as B cell mitogen was obtained from Gibco BRL Laboratories (Grand Island, NY). PMA (Sigma Chemical Co.) and calcium ionophore A23187 (Behring Diagnostics, La Jolla, CA) were used for T cell stimulation.

Three-Color Immunofluorescence Analysis

Expression of CD60 on CD45RO⁺ and CD45RO⁻ populations of CD4⁺ or CD8⁺ T cells was analyzed by the three-color immunofluorescence method as described (19). Heparinized whole blood or PBMC from donors of various ages below were simultaneously stained with FITC-conjugated anti-CD60, PE-conjugated anti-CD45RO, and Per-CP-conjugated anti-CD4 or anti-CD8 mAbs. Subsequently, whole blood samples were then lysed in a 0.83% NH₃Cl lysis buffer. Per-CP-positive (CD4⁺ or CD8⁺) lymphocytes were gated, and the relation between CD60 and CD45RO expressed on CD4⁺ or CD8⁺ T cells were analyzed in a two-color manner on a Cytoron Absolute flow cytometer (Ortho Diagnostic Systems).

Cell Preparation

Neonatal blood samples were collected from the umbilical vein immediately after the uneventful delivery of full-term newborns. Venous blood from healthy infants and children of various ages obtained after the parental informed consent was taken. Adult donors were PPD-reactive healthy volunteers between 22 and 40 years of age. People between 80 and 85 years of age were included as the elderly persons. PBMC were obtained from heparinized whole blood by Ficoll-Hypaque density gradient centrifugation. PBMC were further separated into E-rosetting (E⁺) and nonrosetting (E⁻) cells by rosette formation with 2-aminoethylisothiuronium bromide-treated SRBC, followed by Ficoll-Hypaque gradient centrifugation as described (20).

For isolation of naive (CD45RO⁻) CD4⁺ T cells and CD60⁺ or CD60⁻ subpopulations of memory (CD45RO⁺) T cells, E⁺ cells were stained for CD60 (FITC), CD45RO (PE) and CD4 (Per-CP) in a three-color manner as above. For B cells and monocytes, E⁻ cells were stained in a two-color manner with PE-conjugated anti-CD20 and FITC-conjugated CD14 mAb. Isolation of each cell population was performed by an electronic sorting using an EPICS Elite flow cytometer (Coulter Electronic, Inc., Hi-aleah, FL) as described elsewhere (21). The purity of each

isolated cell population routinely exceeded 95% as assessed by a flow cytometric analysis.

Cell Cultures

The culture medium consisted of RPMI 1640 medium containing 10% FCS, 20 mmol/L Hepes, 5×10^{-5} mol/L 2-ME, 0.3 mg/ml L-glutamine, 200 U/ml penicillin G, and 10 μ g/ml gentamicin. All cultures were performed at 37°C in a humidified incubator of 5% CO₂ and 95% air. For proliferative assay, purified CD4⁺ T cell subpopulations were seeded at 4×10^4 cells/well in 96-well flat-bottom microtiter plates (Corning Glass Works, Corning, NY) together with 10% autologous monocytes (4×10^3 /well) in a final volume of 0.2 ml and incubated with 10 μ g/ml PHA for 3 days or with 1 μ g/ml PPD or irradiated (1500 cGy) allogeneic PBMC (4×10^4 cells/well) for 5 days. DNA synthesis was determined by [³H]TdR incorporation (0.2 μ Ci/well, 6.7 Ci/mmol; New England Nuclear, Boston, MA) during the last 12 h of culture (22). For cytokine production, CD4⁺ T cell subpopulations were cultured at 2×10^5 cells/well in 96-well microtiter plates in 0.2 ml in the presence of 20 ng/ml PMA plus 250 ng/ml calcium ionophore A23187. After 3 days, culture supernatants were collected and stored at -70°C until cytokine assay. For Ig production by B cells, B cells were seeded at a cell density of 2×10^4 /well together with autologous monocytes (6×10^3 /well) and were cultured with or without added CD4⁺ T cell subpopulations (4×10^4 /well) in the presence of a 1:200 dilution of PWM. After 10 days, culture supernatants were harvested for quantitation of Ig levels.

Measurements of Ig Secretion and Cytokine Production

Ig concentrations in culture supernatant were quantitated by using isotype-specific ELISA assay as described (23). The sensitivity of the assay was follows: IgG and IgA, >5 ng/ml; and IgM, >10 ng/ml. The levels of IL-2, IL-4, IL-10, and IFN- γ in culture supernatants were determined by using the commercial ELISA kits (Medgenix Diagnostics, Bruxelles, Belgium) and performed according to the manufacture's instructions. Minimum sensitivities of these assays were >0.1 U/ml for IL-2, >2.0 pg/ml for IL-4, >1.0 pg/ml for IL-10, and >0.03 IU/ml for IFN- γ .

Statistical Analysis

Data were analyzed for statistical significance ($P < 0.05$) using paired Student's *t* test.

RESULTS

Preferential Expression of CD60 on Memory (CD45RO⁺) T Cells

Consistent with previous observations (10, 13), preliminary experiments by two-color analysis indicated

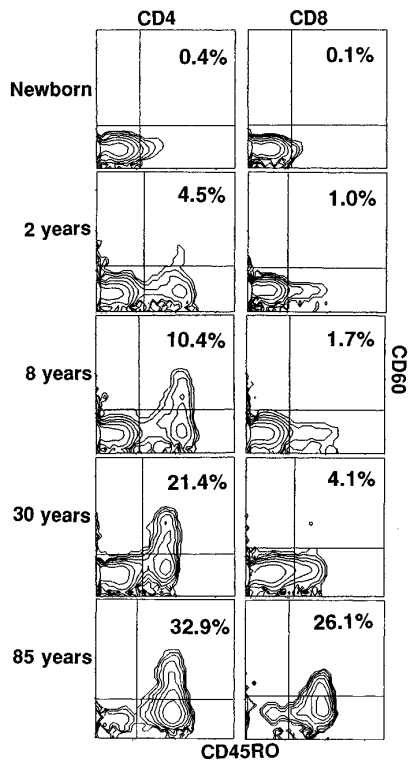


FIG. 1. Preferential expression of CD60 on CD45RO⁺ memory T cells. Heparinized whole bloods from donors of various ages were simultaneously stained with Per-CP-conjugated anti-CD4 or anti-CD8, PE-conjugated anti-CD45RO, and FITC-conjugated anti-CD60 mAbs. Two-color profiles of CD45RO (PE) and CD60 (FITC) expressed on CD4⁺ or CD8⁺ T cells were obtained by gating on red fluorescence (Per-CP) and forward lights scatter to identify corresponding T cells.

that the CD60 antigen was found only on a fraction of CD3⁺ T cells, but the antigen was not detected on CD20⁺ B cells, CD16⁺ NK cells, and CD14⁺ monocytes (data not shown). Human peripheral T cells consist of two major populations, namely naive and memory

cells, which are identifiable by the absence and presence of CD45RO expression, respectively (14–16). We examined whether CD60 expression might be restricted to either of these T cell populations. For this purpose, three-color immunofluorescence analysis was performed to examine the relationship between CD60 and CD45RO expressed on CD4⁺ or CD8⁺ T cells. Figure 1 shows representative profiles obtained from blood samples of various ages. In adult individuals, it was found that CD60 was preferentially expressed on memory (CD45RO⁺) T cells, but, if any, less on naive (CD45RO[−]) T cells. CD60 expression on memory T cells was especially marked for CD4⁺ T cells. CD60 staining is dimmer in the CD8⁺ T cell population. Therefore, it is difficult to distinguish clearly CD60-positive from CD60-negative cells. This may cause an underestimation of the progression of CD8 cells toward increasing CD60 expression with age. In contrast to adults, memory T cells expressing CD60 were rare in the blood of newborns and young children. Table 1 summarizes age-related changes in percentages of memory CD4⁺ and CD8⁺ T cells expressing CD60. A frequency of memory T cells in the peripheral blood expand with increasing antigenic stimuli after birth. As expected, percentages of CD60⁺ memory CD4⁺ T cells inclined to increase gradually with advancing age in parallel with the appearance of CD45RO-defined memory CD4⁺ T cells. It should be noted that despite memory CD8⁺ T cells appearing in the blood as a function of age, CD60 expression on memory CD8⁺ T cells was extremely low until adulthood. Nevertheless, the elderly persons were observed to show appreciable expression of CD60 on memory CD8⁺ T cells.

Proliferative Responsiveness of Memory CD4⁺ T cells with and without CD60 Expression

On the basis of CD60 and CD45RO expression, CD4⁺ T cells from PPD-reactive adult donors were separated

TABLE 1
Age-Related Changes of CD45RO⁺ and CD60⁺ Cells within CD4⁺ or CD8⁺ T Cells

Age	n	% of positive cells in CD4 ⁺ cells		% of positive cells in CD8 ⁺ cells	
		CD45RO ⁺	CD60 ⁺ 45RO ⁺	CD45RO ⁺	CD60 ⁺ 45RO ⁺
Newborn	6	5.4 ± 2.1	0.7 ± 0.3	4.6 ± 1.8	0.3 ± 0.1
0–6 months	6	10.4 ± 2.1	1.4 ± 1.0	6.8 ± 3.8	0.6 ± 0.2
7–11 months	8	19.9 ± 3.0	3.8 ± 2.2	17.3 ± 10.5	1.4 ± 1.2
1–5 years	8	26.7 ± 6.0	7.0 ± 2.8	17.0 ± 7.1	2.2 ± 1.0
6–16 years	10	36.9 ± 10.2	13.4 ± 5.2	22.5 ± 8.6	3.4 ± 1.7
20–40 years	10	51.8 ± 15.5	20.8 ± 6.9	33.5 ± 10.9	3.4 ± 1.7
80–85 years	6	63.6 ± 16.3	27.3 ± 5.2	58.1 ± 17.8	21.1 ± 9.4

Note. Heparinized whole blood from donors of various ages was simultaneously stained with FITC-conjugated anti-CD60, PE-conjugated anti-CD45RO, and Per-CP-conjugated anti-CD4 or anti-CD8 mAb and then lysed by NH₄Cl lysis buffer. The percentages of CD45RO⁺ and CD60⁺CD45RO⁺ cells within CD4⁺ or CD8⁺ T cells were evaluated by three-color immunofluorescence analysis. The data are expressed as the mean ± SEM.

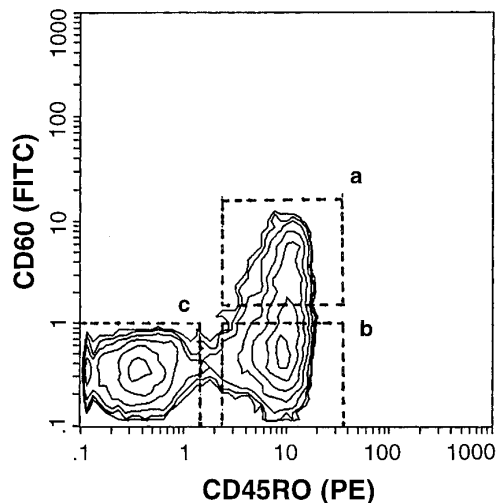


FIG. 2. Three-color immunofluorescent analysis of CD45RO and CD60 expressed on CD4⁺ T cells. E-rosetting T cells from an adult donor were triple stained as described in the legend to Fig. 1. Two-color profiles of CD45RO (PE) and CD60 (FITC) expression on CD4⁺ T cells were obtained using an EPICS Elite flow cytometer. As surrounded by the dashed lines, three subpopulations of CD4⁺ T cells were isolated by using the flow cytometer: (a) CD60⁺ memory (CD45RO⁺) cells, (b) CD60⁻ memory (CD45RO⁺) cells, and (c) naive (CD60⁻CD45RO⁻) cells.

into three cell populations, naive (CD60⁻CD45RO⁻) cells and two memory subsets (CD60⁺CD45RO⁺ and CD60⁻CD45RO⁺) by a cell sorting (Fig. 2). These CD4⁺ T cell subpopulations were tested for the ability to proliferate in response to PPD as a recall antigen, PHA as the polyclonal activator or allogeneic MLC. As shown in Fig. 3, a proliferative responsiveness to PPD as a function of memory cells was evident in CD60⁺ memory CD4⁺ T cells, whereas the response to PPD was markedly feeble in CD60⁻ memory CD4⁺ T cells as well as naive CD4⁺ T cells. In contrast, CD60⁺ memory CD4⁺ T cells proliferated in response to PHA at significantly lower levels than other CD4⁺ T cell

subpopulations. On the other hand, proliferation in response to allogeneic cells occurred similarly in all of CD4⁺ T cell subpopulations.

Cytokine Production by CD60⁺ and CD60⁻ Memory CD4⁺ T Cells

It has been shown that naive CD4⁺ T cells produce only IL-2, whereas memory CD4⁺ T cells are good producers of IL-4, IL-10, and IFN- γ (24–27). We examined whether IL-4, IL-10, and IFN- γ might be reciprocally produced by memory CD4⁺ T cells with and without CD60 expression. For this, the production ability of these cytokines by CD60⁺ and CD60⁻ memory CD4⁺ T cells was evaluated on stimulation with PMA plus calcium ionophore in comparison with naive CD4⁺ T cells. As shown in Fig. 4, all CD4⁺ T cell subpopulations produced substantial levels of IL-2 in response to a potent stimulation with PMA plus calcium ionophore. Reasonably, variable levels of IL-4, IL-10, and IFN- γ were detected in culture supernatants from two subpopulations of memory CD4⁺ T cells, but not from naive ones. Importantly, it was found that levels of IL-4 and IL-10 were significantly higher in culture supernatants from CD60⁺ memory CD4⁺ T cells than with those seen in CD60⁻ memory CD4⁺ T cells. However, IFN- γ levels in culture supernatants were not statistically different between CD60⁺ and CD60⁻ memory CD4⁺ T cells.

Helper Activity of CD60⁺ and CD60⁻ Memory CD4⁺ T Cells for Ig Production by B Cells

Memory CD4⁺ T cells, but not naive ones, have been shown to mediate helper activity for B cell differentiation into Ig-producing cells (23). We examined which of CD60⁺ and CD60⁻ memory CD4⁺ T cells might exert mainly helper function for Ig production by B cells. B cells were cultured with autologous CD4⁺ T cell subpopulations, including naive ones, in the presence of

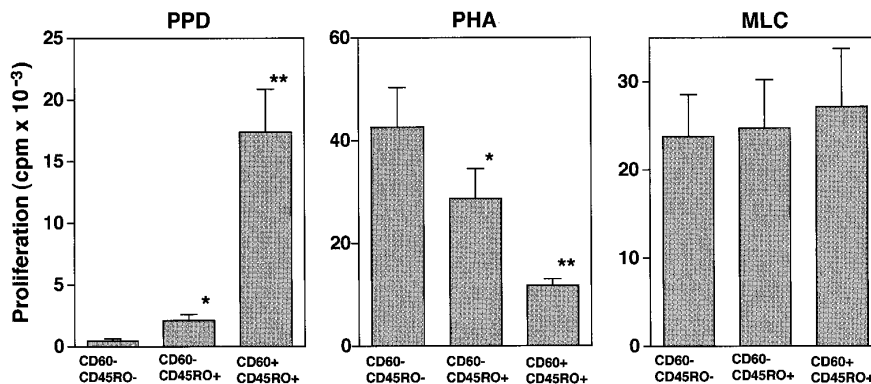


FIG. 3. CD4⁺ T cell subpopulations were cultured with PHA for 3 days and PPD or irradiated allogeneic PBMC for 5 days. Values for [³H]thymidine incorporation are expressed as mean cpm $\pm$ SEM of five experiments. * P < 0.02, ** P < 0.005 compared with CD60⁻CD45RO⁻ subsets and CD60⁻CD45RO⁺ subsets, respectively.

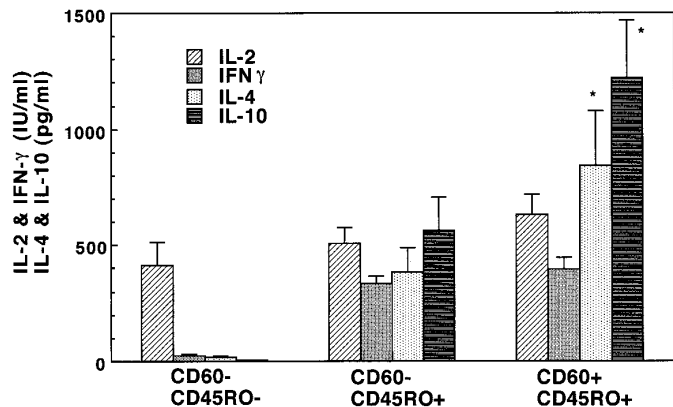


FIG. 4. Cytokine production by CD60⁻ and CD60⁺ memory CD4⁺ T cells. Separated CD4⁺ T cell subpopulations (1×10^6 /ml) were cultured with PMA plus calcium ionophore for 3 days. The levels of IL-2, IFN- γ , IL-4, and IL-10 in culture supernatants were measured by ELISA. The data are expressed as the mean $\pm$ SEM of six independent experiments on six different donors. * $P < 0.03$ in Student's t test compared with cytokine levels produced in CD60⁻CD45RO⁺ subsets.

PWM, and the levels of IgG, IgM, and IgA in culture supernatants were determined. Consistent with previous results, memory CD4⁺ T cells induced production of higher levels of all Ig classes by B cells than with naive ones (Fig. 5) (23). As can be seen in the figure, it was found that CD60⁺ memory CD4⁺ T cells appeared to exhibit more efficient helper activity for Ig production by B cells than CD60⁻ memory CD4⁺ T cells.

DISCUSSION

After the completion of thymic development, Ag-inexperienced or naive T cells are driven by increasing external antigenic exposures after birth to differentiate and mature into Ag-primed cells, a small fraction of which may behave as virtually memory cells with the long-lived nature. Accumulating evidence has presented that Th effector cells can be divided into Th1 and Th2 subsets by types of cytokine produced (28). Th1 cells secrete IFN- γ , lymphotoxin, and IL-2 and mediate delayed hypersensitivity, whereas Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13 and mediate Ig production by B cells. Furthermore, the other subset, termed Th0, has been proposed to produce cytokines of both Th1 and Th2 types, and is thought to be obligatory precursors of Th1 and Th2 cells. Although expression of CD45RO, the smallest isoform of the CD45 molecule, is widely used for identification of human memory T cells (14–16), it is conceivable that CD45RO⁺ memory T cells in the periphery may contain heterogeneous populations, namely, recently Ag-primed, active effector or long-lived memory cells, presumably reflecting their activation or differentiation stages after antigenic priming. These memory T cells show enhanced levels of expression of various acces-

sory molecules, all of which serve to activate T cells, possibly through the cell–cell adhesion (14). However, a suitable marker for discrimination of the heterogeneity of CD45RO⁺ memory T cells is not yet available.

In light of previous observations that CD60 expression appears to identify a functional subset of human T cells (8, 10, 12, 13), our interests were directed at elucidation of whether CD60 expression might be present on a functionally specified population of CD45RO⁺ memory T cells. In this work, the three-color immunofluorescence method was used to examine developmental changes of CD60 expression on naive (CD45RO⁻) and memory (CD45RO⁺) populations of CD4⁺ and CD8⁺ T cells. The three-color analysis clearly revealed that the CD60 antigen was preferentially expressed on memory (CD45RO⁺) T cells, but was undetectable on naive (CD45RO⁻) T cells. As expected, CD60⁺ T cells was negligible in the neonatal blood and increased with advancing age in parallel to the appearance of CD45RO⁺ memory T cells in the blood (29). The important point was that CD60 expression was restricted to a defined proportion of memory T cells throughout the whole period of human life. It would be necessary to confirm whether the functions of phenotypically similar subsets from subjects of different ages are equal or not. Previous studies have described that CD60 is expressed on a subset of CD8⁺ as well as CD4⁺ T cells (13), whereas our analysis showed that CD60 expression on memory T cells was prominent for CD4⁺ cells, but, if any, less for memory CD8⁺ T cells. CD60 expression on memory CD8⁺ T cells was noted only in elderly individuals.

Using a cell-sorting technique, we separated memory (CD45RO⁺) CD4⁺ T cells from PPD-sensitive adult

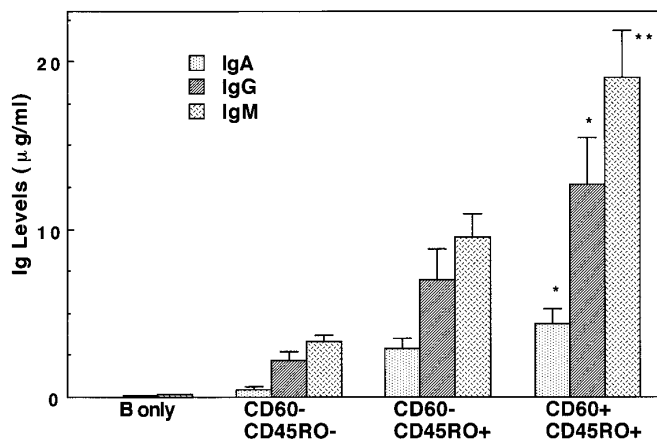


FIG. 5. Helper activity of CD60⁻ and CD60⁺ memory CD4⁺ T cells for Ig production by B cells. CD20⁺ B cells (2×10^4 /well) were combined autologous monocytes (6×10^3 /well) and cultured alone or with CD60⁻CD45RO⁻, CD60⁻CD45RO⁺, or CD60⁺CD45RO⁺CD4⁺ T cells (4×10^4 /well) in the presence of PWM. Supernatants were collected on day 10 for quantitation of Ig secretion by ELISA. The data are expressed as the mean $\pm$ SEM for five experiments. ***Significant difference in Ig levels compared with CD60⁻CD45RO⁺ CD4⁺ T cells (paired Student's t test, * $P < 0.05$, ** $P < 0.01$).

donors into two (CD60⁺ and CD60⁻) populations on the basis of CD60 expression. Unlike naive (CD45RO⁻) T cells, it has been shown that memory (CD45RO⁺) CD4⁺ T cells are capable of proliferating well in response to the recall antigen (22), mediating helper activity for Ig production by B cells (23) and producing cytokines such as IL-4, IL-10, and IFN- γ (25). These functional properties were compared among memory CD4⁺ T subpopulations defined by CD60 expression. We found that CD60⁺ memory CD4⁺ T cells accounted for a large part of proliferative responsiveness to the recall antigen as memory cells. In addition, CD60⁺ memory CD4⁺ T cells provided a more efficient helper function for Ig production by B cells than CD60⁻ ones. With respect to cytokine production, CD60⁺ memory CD4⁺ T cells tended to produce higher levels of IL-4 and IL-10 than those with CD60⁻ ones, whereas IFN- γ production was similarly found in both memory CD4⁺ T cell subpopulations. Since it has been shown that Th1- and Th2-type cytokines induced IgG2a and IgG1 subclass, respectively, in mice, further analysis of the IgG subclass profile produced by B cells in the presence CD60⁺ subpopulations would be necessary.

A possibility that CD60 might be a kind of activation antigen is unlikely because potent stimulation with PHA failed to induce CD60 expression on activated (CD45RO⁺) CD4⁺ T cells, whereas the cultures with the recall antigen resulted in selective expansion of CD4⁺ T cells with CD60 expression (data not shown). We also observed that activated T cells in EBV-induced infectious mononucleosis expressed CD45RO as well as HLA-DR antigens as a sign of *in vivo* activation (30), but they never exhibited CD60 expression (unpublished observations). On the analogy of results of functional studies here, it is tempting to suppose that CD60⁺ memory CD4⁺ T cells in the blood might represent the effector population with Th2-like function in the *in vivo* situation (27). Studies on human CD4⁺ Th clones have showed that Th2 clones promote a generation of Ig-producing cells by B cells (31). It has been reported that the CD60⁺ subset of CD8⁺ T cells from the peripheral blood helps Ig production by B cells and favors IL-4 production (13). Seemingly supporting our idea, cytokine production studies showed that CD60⁺ memory CD4⁺ T cells produced higher levels of IL-4 and IL-10 than CD60⁻ ones. Semnani *et al.* reported that repeated costimulation of CD4⁺ memory T cells with LFA-3 led to secretion of high levels of IL-5 and low levels of GM-CSF, and maintained significant levels of CD60 expression cells, suggesting a positive correlation between CD60 expression and a Th2 cytokine profile (32). However, the clearly detectable levels of IFN- γ after LFA-3 costimulation differ from the usual Th2-like cytokine pattern. Our results also showed that IFN- γ production by CD60⁺ memory CD4⁺ T cells was almost equivalent to that seen in their counterparts. Thus, a possibility is raised that CD60 may be

expressed on both populations producing Th1-like and Th2-like cytokines within memory CD4⁺ T cells. Regarding this, our other study disclosed that the majority of memory CD4⁺ T cells expressed L-selectin and produced Th2-type cytokines, whereas a minority of them lacked L-selectin expression and secreted Th1-type cytokines (33). In our laboratory, preliminary four-color analysis indicated that CD60 expression was appreciably found on both L-selectin-positive and -negative populations of memory CD4⁺ T cells. Furthermore, it is shown that CD4⁺CD45RO⁺CD27⁻ T cell subsets contains specialized primed T cells as IL-4- and IL-5-secreting capacity (34). Approximately only 30–40% of CD45RO⁺CD60⁺ cells were CD27⁻ cells (data not shown), suggesting that CD45RO⁺CD60⁺ and CD45RO⁺CD27⁻ cells were a different population. Further study will be necessary to resolve the function of CD45RO⁺CD60⁺CD27⁺.

Taken together, it seemed that the CD60 antigen might be essentially expressed on the functional differentiated memory effector cells, whether with Th1- or Th2-like functions. Recent studies on Th clones have disclosed that expression of CD30 might be associated with Th2 function (35–37). Del Prete *et al.* (38) have made an intriguing report that a few circulating CD30⁺CD4⁺ T cells in atopic patients appeared to actually produce Th2-type cytokines. At present, an alteration in the Th1/Th2 response has been implicated in the pathogenesis of several pathological conditions such as parasitic infections, atopic diseases, HIV infection, and autoimmune diseases (39, 40). With the combined use of CD60 expression with other markers such as CD30, further investigations on clinical samples from these diseases would allow for understanding the immunological significance of CD60 expressed on memory T cells in the development and differentiation of human T cells.

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