Role of Interleukin 6 for Differential Responsiveness of Naive and Memory CD4+ T Cells in CD2-mediated Activation

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Summary

The present study was undertaken to elucidate different requirements for CD2-mediated activation of naive ($CD45RO^-$) and memory ($CD45RO^+$) $CD4^+$ T cells. A mitogenic combination of anti-CD2 (anti-$T11_2$ and anti-$T11_3$) mAbs could effectively induce the proliferation of memory $CD4^+$ T cells even in the absence of monocytes. In marked contrast, naive $CD4^+$ T cells did not disclose any proliferative responses to anti-CD2 mAbs, when monocytes were absent in culture. This differential responsiveness of naive and memory $CD4^+$ T cells appeared to be related largely to a difference in IL-6–producing ability between both populations. IL-6 among monocyte-derived cytokines could correct unresponsiveness of naive $CD4^+$ T cells to anti-CD2 stimulation. Unlike naive $CD4^+$ T cells, memory $CD4^+$ T cells produced IL-6 by themselves, with its mRNA being expressed on anti-CD2 stimulation. Anti-IL-6R mAb significantly inhibited proliferation of memory $CD4^+$ T cells seen in the anti-CD2–stimulated cultures without monocytes, indicating the involvement of their own production of IL-6 in CD2-mediated activation. The results suggest an essential role of IL-6 for triggering of $CD4^+$ T cells via the CD2 molecule.

The 50-kD CD2 molecule, originally defined as the sheep erythrocyte receptor, is one of the earliest T cell differentiation antigens to appear on developing thymocytes before the stage of cell surface expression of TCR-CD3 (1, 2). In addition to the TCR-CD3 complex, a number of studies have indicated that the CD2 molecule can serve to activate human T cells (3–5). The cDNA encoding human CD2 has been cloned and the predicted structure of CD2 reveals a transmembrane glycoprotein with a large cytoplasmic domain (126 amino acids), suggesting that it could function in signal transduction in T cells (6). More recently, lymphocyte function-associated antigen-3 (LFA-3), which is expressed on a broad spectrum of hematopoietic and nonhematopoietic cells, has been identified as a natural ligand for CD2 in man (7). It has been demonstrated that a combination of mAbs directed against two distinct epitopes of CD2, namely $T11_2$ and $T11_3$, successfully triggers proliferation of and lymphokine production by peripheral T cells (3, 8). Although the physiological role of CD2 in thymic ontogeny or in immune responses has been proposed, the whole sequence of events involved in T cell activation via CD2 is not clearly understood.

Recent studies have indicated that the naive and memory cell populations within human T cells can be distinguished phenotypically by surface markers (9–13). Isoforms of CD45 (CD45RO or CD45RA) are useful markers for identification of both populations. Expression of CD45RO identifies human memory T cells, while human naive T cells are confined to the $CD45RA^+$ subset. The results of studies using these markers have disclosed some functional differences between memory and naive T cells, including production of cytokines such as IL-4 or IFN-γ (10, 14), and CD3- or CD2-mediated activation (15–17), in addition to a difference in responses to recall antigens. In this report, we document a marked difference between naive ($CD45RO^-$) and memory ($CD45RO^+$) $CD4^+$ T cells in responsiveness to anti-CD2 stimulation. Memory $CD4^+$ T cells were able to respond to a pair of anti-CD2 mAbs (anti-$T11_2$ and anti-$T11_3$) to proliferate even in the absence of monocytes, whereas any proliferative responses induced by anti-CD2 mAbs were not observed in naive $CD4^+$ T cells. The presence of monocytes in cultures resulted in induction of anti-CD2–induced proliferation of naive $CD4^+$ T cells. It was significant that IL-6 could correct unresponsiveness of naive $CD4^+$ T cells to anti-CD2 stimulation. Unlike naive $CD4^+$ T cells, memory $CD4^+$ T cells appeared to utilize their own producing IL-6 for proliferation upon anti-CD2 stimulation. The results suggest an essential role of IL-6 in initiation of activation of $CD4^+$ T cells through the CD2 molecule.

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Materials and Methods

Cytokines. Human rIL-6 was a kind gift of Drs. T. Hirano and T. Kishimoto (Osaka University, Osaka, Japan). Preparations of rIL-6 had a specific activity of 5×10^6 U/mg of protein, determined by the enhancement of IgM production by an EBV-transformed line (SKW6-C14 cells) (18). Human rIL-1β (a gift of Dr. Y. Hirai, Otsuka Pharmaceutical Co., Tokushima, Japan) had a specific activity of 2×10^7/mg of protein, estimated by the mouse thymocyte proliferation assay. Human rIL-2 was obtained from Shionogi & Co. (Osaka, Japan) and human rTNF-α was from Suntory (Osaka, Japan).

Monoclonal Antibodies. Anti-CD2 mAbs, anti-$T11_2$ (IgG2a), and anti-$T11_3$ (IgG3) were kindly supplied in the ascites forms by Dr. E. L. Reinherz (Dana Farber Cancer Institute, Boston, MA). Two mAbs against human IL-6R, PM1 (IgG1), and MT18 (IgG2b), which have recently been developed (19), were generously provided by Drs. T. Taga and T. Kishimoto (Osaka University, Osaka, Japan). One, PM1, inhibits the IL-6-dependent cellular proliferation. The other, MT18, is not blocked by IL-6 for its recognition of IL-6R; therefore, it may be suitable for immunostaining of cells. Anti-Tac mAb (IgG2a) against human IL-2R (CD25) (20) was a kind gift of Dr. T. A. Waldmann (National Institutes of Health, Bethesda, MD). The mAb UCHL1 (IgG2a) against CD45RO (12) was purchased from Dakopatts A/S (Copenhagen, Denmark). FITC-conjugated anti-CD4 (anti-Leu-3a, IgG1) mAb was from Becton Dickinson Immunocytometry Systems (Mountain View, CA). The KNT-4 mAb (IgG2a), which has been originally prepared as the antibody against rIL-2, but is not reactive with the natural form of IL-2 (21), served as the control antibody.

Cell Preparation. PBMC were obtained from adult volunteers between the ages of 25 and 32 yr by Ficoll-Hypaque density gradient centrifugation. E-rosetting T cells were separated from non-rosetting cells by rosetting with 2-aminoethylisothiouronium bromide–treated SRBC, followed by Ficoll-Hypaque density gradient centrifugation (22). After lysis of rosetted SRBC, T cells from the pellet were further depleted of contaminated monocytes by two cycles of plastic adherence. Subsequently, purified T cells were stained for CD45RO and CD4 by the two-color immunofluorescence method, and separated into $CD45RO^-$ (naive) and $CD45RO^+$ (memory) populations of $CD4^+$ T cells by using an Epics-C flow cytometer (Coulter Electronics, Inc., Hialeah, FL) as described (23). The resulting T cell populations contained <0.001% esterase-positive monocytes. Adherent cells as the source of monocytes were obtained from PBMC on plastic flasks and irradiated with 3,000 rad.

Cell Cultures. The culture medium consisted of RPMI 1640 (Gibco Laboratories, Grand Island, NY) containing 10% heat-inactivated pooled human serum, 25 mM Hepes, 5×10^{-5} M 2-ME, 0.3 mg/ml L-glutamine, 200 U/ml penicillin G, and 10 μg/ml gentamicin. All cultures were set up in 96-well flat-bottomed plates (Corning Glass Works, Corning, NY) in a final volume of 200 μl of culture medium. The plates were incubated at 37°C in a humidified atmosphere of 5% CO_2. For proliferative assays, the cells were seeded at 3×10^4/well and stimulated for 72 h with a combination of anti-$T11_2$ and anti-$T11_3$ mAbs alone or together with monocytes or cytokines. DNA synthesis was determined by [^{3}H]TdR incorporation (0.2 μCi/well, 6.7 Ci/mmol; New England Nuclear, Boston, MA) during the last 12 h of culture. In some experiments, the cells were stimulated with 0.1% (vol/vol) PHA-P (Difco Laboratories, Detroit, MI) and 10 ng/ml PMA (Sigma Chemical Co., St. Louis, MO) to evaluate induction of IL-6 activity and IL-6 mRNA by T cells. In addition, LPS (1 μg/ml; Difco Laboratories) was used to examine contamination of monocytes/macrophage lineage cells in isolated $CD4^+$ T cell populations. The cells were cultured at 1×10^5/well for determination of IL-6 mRNA expression or IL-2 secretion and at 5×10^5/well for demonstration of IL-6 activity in culture supernatants, as described below.

IL-2 Assay. IL-2 was measured by the ability to support the growth of murine IL-2-dependent CTLL-2 cells as described previously (24). IL-2 activity was evaluated by comparison with an IL-2 standard preparation (Biological Response Modifiers [BRM] standard).

IL-6 Assay. IL-6 activity in culture supernatants was determined by a colorimetric assay using an IL-6-dependent murine hybridoma clone, MH60.BSF2, as described elsewhere (25). 1 U of IL-6 was here arbitrarily defined by the amount required for 50% of the maximal response of the hybridoma cells.

IL-6 mRNA Analysis by Polymerase Chain Reaction. Expression of IL-6 mRNA was evaluated by amplification using PCR as described (26). $CD4^+$ T cell subpopulations were harvested after 12 h of incubation in various culture conditions. Total cellular RNA was isolated by the acid guanidinium-phenol-chloroform method (27), following the addition of 3 μg of yeast tRNA as a carrier to harvested cells. First strand DNA was synthesized at 42°C for 1 h in a final volume of 50 μl reaction mixture; RNA from 2×10^5 cells in 50 mM Tris-HCl, pH 8.3, 100 mM KCl, 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM each dNTP, 40 μg/ml oligo-dT (Pharmacia LKB Biotechnology, Bromma, Sweden), and 10 U of reverse transcriptase (RAV-2; Takara Shuzo Co., Ltd., Kyoto, Japan). After incubation, the products were passed through Sephadex G-50 spun column and were used as first strand cDNA for PCR amplification.

85 μl of PCR mixture was added to 5 μl of first strand cDNA. PCR mixture contained 53.5 μl sterile water, 10 μl 10× reaction buffer, 16 μl of dNTP mix (each at 1.25 mM), and 0.5 μl (2.5 U) of Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT). PCR reaction buffer (10×) contained 500 mM KCl, 100 mM Tris-HCl, pH 8.3, 20 mM $MgCl_2$, and 0.01% gelatin. 5 μl of each primer was added to give a final concentration of 1 mM. The primers used were ATGAACTCCTTCTCCACAAGC and CTACATTTGCCGAAGAGCCCTCAG and were 594 bp apart (18). Amplified fragments of 639 bp were thus expected. The mixture was subjected to PCR amplification using a DNA Thermal Cycler (Perkin-Elmer Cetus). Denaturation was performed at 94°C for 1.5 min, annealing at 55°C for 1.5 min, and primer extension at 72°C for 2 min. These steps were repeated 30 times. 10 μl of each amplified product was electrophoresed through 1.3% agarose gels to separate PCR fragments, which were transferred to a nitrocellulose membrane and hybridized with IL-6 DNA probe (provided by Drs. T. Hirano and T Kishimoto) labeled with digoxigenin (Boehringer Mannheim Biochemicals, Indianapolis, IN). The TaqI-XbaI fragments of IL-6 cDNA were used as a probe (18). The immunoenzymic detection of hybridized DNA was performed according to a recommendation of the manufacturer.

Results and Discussion

A Marked Difference between Naive and Memory $CD4^+$ T Cells in Proliferative Responsiveness to Stimulation with Anti-CD2 mAbs. A mitogenic combination of anti-CD2 (anti-$T11_2$ and anti-$T11_3$) mAbs could stimulate effectively proliferation of memory ($CD45RO^+$) $CD4^+$ T cells even in the absence of monocytes (Fig. 1 *B*). In marked contrast, naive ($CD45RO^-$) $CD4^+$ T cells did not exhibit any discernible proliferative responses in a wide range of anti-CD2 mAbs

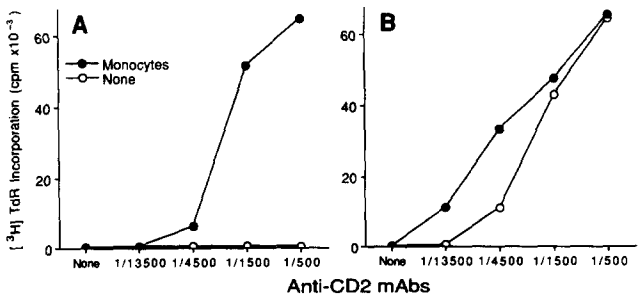

Figure 1. Anti-CD2-induced proliferation of naive (*A*) and memory (*B*) CD4+ T cells in the presence or absence of monocytes. Each CD4+ subpopulation was cultured (3 × 10⁴/well) for 3 d with a combination of anti-CD2 mAbs at various dilutions of ascitic fluids. 10% irradiated monocytes were added at the beginning of culture. [³H]TdR incorporation was measured during the final 12 h of culture. Data are expressed as the mean cpm of triplicate cultures.

Table 1. *Effects of Cytokines on Proliferative Responses of Naive CD4+ T Cells to Anti-CD2 mAbs*

Anti-CD2	Additives	Amount	[³H]TdR incorporation
−	None		424 ± 132
+	None		379 ± 110
+	Monocytes	10%	52,216 ± 3,930
+	IL-2	1 ng/ml	51,357 ± 4,129
+	IL-1β	1 ng/ml	462 ± 82
+	IL-1β	10 ng/ml	433 ± 128
+	TNF-α	1 ng/ml	621 ± 161
+	TNF-α	10 ng/ml	8,534 ± 290
+	IL-6	1 ng/ml	47,155 ± 1,632
+	IL-6	10 ng/ml	51,338 ± 3,425

Isolated naive CD4+ T cells (3 × 10⁴/well) were cultured in the presence of a combination of anti-T11₂ and anti-T11₃ mAbs (1/1,000 dilutions) for 3 d with or without monocytes or cytokines. [³H]TdR incorporation was measured during the last 12 h of culture and expressed as the mean cpm ± SEM of six donors.

examined, when monocytes were absent in culture (Fig. 1 *A*). Importantly, the addition of monocytes to the anti-CD2-stimulated cultures of naive CD4+ T cells resulted in induction of their proliferative responses at the levels comparable to those of memory CD4+ T cells. The restored responsiveness of naive CD4+ T cells to anti-CD2 mAbs was inducible by the addition of at least 3% monocytes (data not shown). It should be noted that the dose-response curves for anti-CD2 mAbs in proliferation of memory CD4+ T cells were never shifted between the presence and absence of monocytes in cultures. These results indicated that, while memory CD4+ T cells might have the ability to respond to appropriate pairs of anti-CD2 mAbs by themselves, naive CD4+ T cells could be triggered by anti-CD2 stimulation to proliferate with help of some signals provided by monocytes.

IL-6 Corrects Unresponsiveness of Naive CD4+ T Cells in CD2-mediated Triggering through IL-2 Production. Generally, activation of T cells in response to antigens, mitogens, or antireceptor antibodies requires the presence of accessory signals provided by the monocyte/macrophage lineage cells. These accessory cells also provide costimulatory cytokines such as IL-1 for T cell stimulation, in addition to the promotion of cell adhesion through MHC-antigen complexes or other surface molecules (28). In the present study we asked whether some monocyte-derived cytokines might play the key role for restoration of unresponsiveness of naive CD4+ T cells to CD2-mediated triggering. The effects of three major products such as IL-1β, TNF-α, and IL-6 on CD2-mediated activation of naive CD4+ T cells were examined in comparison to those of monocytes or IL-2 as T cell growth factors (Table 1). Favorable proliferation of naive CD4+ T cells was observed in the anti-CD2-stimulated cultures with IL-2 (1 ng/ml). With respect to effects of monocyte-derived cytokines, IL-1β did not induce the responsiveness of naive CD4+ T cells to stimulation with anti-CD2 mAbs, although the marginal proliferation was observed at high doses (10 ng/ml) of TNF-α. The most striking finding was that IL-6 could be substituted seemingly for monocytes in correction of unresponsiveness of naive CD4+ T cells to anti-CD2 stimulation. As shown in Fig. 2, dose-response studies for IL-6 showed that the small amount of IL-6 (0.1–0.3 ng/ml) was sufficient for the induction of responsiveness of naive CD4+ T cells to triggering via CD2. On the other hand, the cultures with IL-6 alone did not elicit the proliferation of naive CD4+ T cells. As shown in Table 2, the presence of monocytes in anti-CD2-stimulated cultures resulted in the secretion of the great amount of IL-2 in both naive and memory populations. It was shown that IL-6, but neither IL-1β nor TNF-α, induced the release of IL-2 in naive CD4+ T cells stimulated with anti-CD2 mAbs. These results suggested a major role of IL-6 for anti-CD2-induced proliferation of naive CD4+ T cells corrected by the supplement of monocytes.

Autocrine Secretion and Requirement of IL-6 for CD2-mediated Activation of Memory CD4+ T Cells. Recently, cDNA for the human IL-6R has been cloned (29), and mAbs against the human IL-6R have been developed using murine transfectant cells expressing the human IL-6R (19). Hirata et al. (19), using anti-IL-6R mAb, have demonstrated that IL-6R is

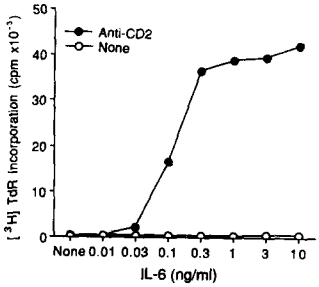

Figure 2. Effect of various doses of IL-6 on proliferation of naive CD4+ T cells in the presence or absence of anti-CD2 mAbs. Naive CD4+ T cells (3 × 10⁴/well) were cultured for 3 d with or without 1/1,000 dilutions of combined anti-CD2 mAbs at various concentrations of IL-6. [³H]TdR incorporation was measured during the final 12 h of culture. Data are expressed as the mean cpm of triplicate cultures.

Table 2. *IL-6 Induces IL-2 Secretion in Anti-CD2-stimulated Naive CD4+ T Cells*

Stimuli	Additives	IL-2 Produced (U/ml) Naive CD4+	Memory CD4+
None	None	<0.03	<0.03
Anti-CD2	None	<0.03	0.48
Anti-CD2	Monocytes (10%)	15.1	8.2
Anti-CD2	IL-6 (1 ng/ml)	0.56	ND
Anti-CD2	IL-1β (1 ng/ml)	0.06	ND
Anti-CD2	TNF-α (1 ng/ml)	0.07	ND

CD4+ T subpopulations were incubated at a concentration of 5 × 10^5 cells/ml in the presence of anti-$T11_2$ and anti-$T11_3$ mAbs (1/1,000 dilution) with monocytes or cytokines. Culture supernatants were collected after 36 h of incubation, and IL-2 activity was measured by the ability to support the growth of CTLL-2.

already expressed on unstimulated CD4+ and CD8+ T cells, although monocytes express much more IL-6R. From immunofluorescence analysis using an anti-IL-6R (MT18) mAb, we found that the expression of IL-6R was seen on both naive and memory CD4+ T cells freshly isolated from the peripheral blood (data not shown). Along with the finding that IL-6 could correct unresponsiveness of naive CD4+ T cells to anti-CD2 stimulation, the constitutive expression of IL-6R on memory as well as naive CD4+ cells seemed to imply to us that IL-6 might operate in CD2-mediated activation of memory CD4+ T cells in the cultures without monocytes as well.

We used an IL-6-dependent murine hybridoma clone, MH60.BSF2, to examine IL-6 activity in culture supernatants from memory and naive CD4+ T cells. Although PHA plus PMA could promote induction of IL-6 activity by both naive

Table 3. *Demonstration of IL-6 Activity in Supernatants from Anti-CD2-stimulated Cultures of Memory but not Naive CD4+ T Cells*

Stimulation	IL-6 activity Naive CD4+	Memory CD4+
	U/ml	
None	<0.2	<0.2
Anti-CD2	<0.2	4.7 ± 1.2
PHA plus PMA	17.3 ± 3.8	30.9 ± 6.1

CD4+ T subpopulations were incubated at a concentration of 2.5 × 10^6 cells/ml with a combination of anti-$T11_2$ and anti-$T11_3$ mAbs (1/500 dilutions) or PHA plus PMA. Culture supernatants were collected after 36 h of incubation, and IL-6 activity was measured by the growth of IL-6-dependent murine hybridoma clone, MH60.BSF2.

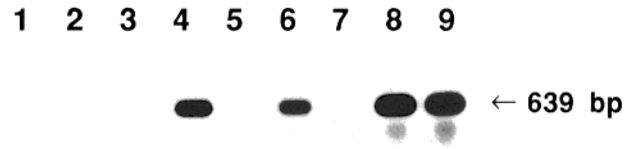

Figure 3. IL-6 mRNA analyzed by PCR amplification. Naive (lanes *1–4*) and memory (lanes *5–8*) CD4+ T cell populations were cultured with various stimulants for 12 h. As described in Materials and Methods, amplified products of IL-6 mRNA were prepared, electrophoresed, transferred onto a nitrocellulose membrane, and hybridized with IL-6 cDNA. The amount of applied samples of LPS-stimulated PBMC (lane *9*) as a control was equivalent to one-fifth of those of CD4+ T cells. Stimulated culture conditions are as follows. (Lanes *1* and *5*) medium; (lanes *2* and *6*) anti-CD2 mAbs; (lanes *3* and *7*) LPS; (lanes *4* and *8*) PHA plus PMA (lane *9*) PBMC stimulated with LPS.

and memory CD4+ T cells, there is a marked difference between both populations in their IL-6 production by anti-CD2 stimulation (Table 3). Stimulation with a combination of anti-$T11_2$ and $T11_3$ mAbs resulted in relatively low but substantial levels of IL-6 activity in supernatants from memory CD4+ T cells. In contrast, IL-6 activity was negligible in supernatants from anti-CD2-stimulated naive CD4+ T cells. Furthermore, the expression of IL-6 mRNA was examined by the amplification method using PCR. Fig. 3 shows the appreciable expression of IL-6 mRNA, apparently being accompanied by the IL-6 secretion, in memory CD4+ T cells stimulated with anti-CD2 mAbs, but not naive CD4+ T cells. Although IL-6 can be produced by a variety of cells, including T cells or B cells, monocytes are undoubtedly the major producers in the peripheral blood (30). Thus, it is possible that restricted production of IL-6 by memory CD4+

Table 4. *Anti-IL-6R mAb Inhibits Proliferative Responses of Memory CD4+ T Cells Induced by Anti-CD2 mAb in the Absence of Monocytes*

Antibody	Amount	[3H]TdR incorporation Exp. 1	Exp. 2	Exp. 3
	μg/ml		*cpm*	
None		27,083	19,154	38,654
Control	20	18,412 (68)*	18,154 (95)	32,612 (84)
Anti-IL-2R	20	491 (2)	1,286 (7)	1,564 (4)
Anti-IL-6R	20	3,931 (15)	2,164 (11)	6,264 (16)
	7	8,661 (32)	9,480 (49)	8,521 (22)
	3	17,747 (66)	12,156 (63)	25,403 (66)

Memory CD4+ T cells (3 × 10^4/well) were cultured in the presence of anti-$T11_2$ and anti-$T11_3$ mAbs (1/1,000 dilutions) without added monocytes for 3 d. Anti-IL-6R (PM1), anti-IL-2R (anti-Tac), or control (KNT-4) mAbs were added at the beginning of the culture. [3H]TdR incorporation was measured during the last 12 h of culture. Data represent the means of triplicate cultures.

* Percent of the responses without the antibody shown in parentheses.

T cells might be due to some contamination of monocytes in these cells. However, this possibility is unlikely, because LPS, a potent stimulator for monocytes, which prompted IL-6 mRNA expression by unfractionated PBMC, did not induce any band of IL-6 mRNA demonstrable by PCR technique in the isolated memory $CD4^+$ T cell population (Fig. 3).

To confirm directly that IL-6 produced by memory $CD4^+$ T cells might be involved in their initiation of CD2-mediated activation, we finally examined whether anti-IL-6R mAb (PM1) with neutralizing ability could inhibit proliferative responses of memory $CD4^+$ T cells without monocytes to a pair of anti-CD2 mAbs (Table 4). As can be expected, anti-IL-2R (anti-Tac) mAb abolished almost completely proliferative responses of memory $CD4^+$ T cells seen in anti-CD2-stimulated cultures without monocytes. Most importantly, it was demonstrated that anti-IL-6R (PM1) mAb exerted unequivocally inhibitory effects, though somewhat less than those by anti-IL-2R, on anti-CD2-induced proliferation of memory $CD4^+$ T cells. Taken together, it appears that the differential responsiveness of naive and memory $CD4^+$ T cells to CD2-mediated triggering would be largely attributable to a difference in IL-6-producing ability between both populations.

Earlier studies have indicated that activation of T cells via the CD2 molecule, unlike stimulation with mitogens, antigens, or antireceptor antibodies such as anti-CD3 or anti-TCR mAbs, appears to be monocyte independent (3). Although most studies on CD2-mediated activation of T cells have been done using peripheral T cells that consist of a mixture of naive and memory cells, a difference between naive and memory T cells in proliferative responses to anti-CD2 antibodies has been reported from several laboratories (16, 17, 31). However, the results appear to be somewhat conflicting. Consistent with the results of this work, some studies have demonstrated enhanced responsiveness of memory ($CD45RO^+$) T cells to anti-CD2 mAbs (16, 17). Alternatively, other studies have indicated that naive ($CD45RA^+$) T cells responded to anti-CD2 stimulation better than memory ($CD45RA^-$) T cells (31). This discrepancy might be, in part, due to different experimental protocols, including used antibodies and cell isolation procedures (electronic cell sorting or panning method). Virtually all T cells from the newborn have been considered to be composed of cells with the so-called naive phenotypes ($CD45RA^+$, $CD45RO^-$) because of paucity of antigenic exposure (32, 33). In other experiments, we found that $CD4^+$ T cells from the neonatal blood were not able to proliferate in response to a pair of anti-$T11_2$ and anti-$T11_3$ mAbs, but their proliferation in response to anti-CD2 stimulation was inducible by the addition of autologous monocytes (unpublished observations). Gerli et al. (34) have also described extremely low responses of neonatal T cells to anti-CD2 antibodies. Regarding whether naive T cells may actually fail to proliferate in response to atni-CD2 mAbs, a report by Fox et al. (35) is of particular importance, demonstrating that anti-CD2 ($T11_2$ and $T11_3$) mAbs alone are ineffective for stimulation of thymocytes. Thymocytes could proliferate well in the presence of both anti-CD2 mAbs and IL-2. In view of these observations, it is feasible to suppose that antigen-unprimed or naive T cells may require additional signals such as monocyte-derived or T cell–derived factors for CD2-mediated activation.

In conclusion, action of IL-6, whether autocrine or paracrine, may be crucial for CD2-mediated activation of $CD4^+$ T cells. Based on the idea that the CD2 molecule plays a role in early thymic ontogeny, the finding that acquisition of the ability to produce IL-6 on CD2-mediated activation occurs only after priming with antigens may have some implications for the understanding of intrathymic and extrathymic differentiation of human T cells.

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