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Research Article

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Cellular and Molecular Basis of Human $\gamma\delta$ T Cell Activation

Role of Accessory Molecules in Alloactivation

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Abstract

Although $\gamma\delta$ T cell receptor-bearing lymphocytes ($\gamma\delta$ T cells) constitute a significant minority of circulating and tissue-associated T lymphocytes, the mechanism responsible for the activation of these cells is unknown. To address this question, resting $\gamma\delta$ TCR+, CD3+, CD4-, CD8- cells isolated from the blood of healthy volunteers were cultured with allogeneic dendritic cells (DC) or monocytes, and their proliferative response measured. DC alone induced $\gamma\delta$ T cells to proliferate, with a peak response on the sixth day of culture. Pretreatment of DC with an anti-HLA-DR mAb, but not anti-HLA class I or anti-CD1 mAbs, inhibited the response of $\gamma\delta$ T cells. Antibodies to $\gamma\delta$ T cell receptor, CD2, CD3, or CD11a were also inhibitory, whereas antibodies to $\alpha\beta$ T cell receptor, CD4, CD5, and CD8 had no effect. Although only 40–60% of freshly isolated $\gamma\delta$ T cells expressed CD28, mAbs directed against CD28 or its ligand, CD80, were markedly inhibitory. Moreover, removal of CD28+ cells from the $\gamma\delta$ T cell population nearly abrogated the response to DC. These results demonstrate that resting $\gamma\delta$ T cells recognize and respond to MHC class II determinants on allogeneic DC in a manner that is highly dependent on the CD28 activation pathway as well as molecules such as CD2 and CD11a that mediate cell-to-cell adhesion. (*J. Clin. Invest.* 1995; 95:296–303.) Key words: dendritic cells • CD28 • HLA-DR • CD2 • CD11a

Introduction

$\gamma\delta$ T cell receptor-bearing lymphocytes ($\gamma\delta$ T cells)¹ constitute up to 10% of the peripheral blood T lymphocytes in man and are also found in substantial numbers within spleen, lymph nodes, and intestine (1, 2). They accumulate in the skin lesions of patients with leprosy or cutaneous leishmaniasis (3) and in the peripheral blood of patients with leishmania (4), malaria (5), Epstein Barr virus infection (6), and HIV infections (7, 8). Individual $\gamma\delta$ T cell lines and clones have been reported to

recognize a variety of antigens including MHC class I (9–11), MHC class II (12, 13), CD1 (14, 15), and bacterial products such as mycobacterial proteins (3, 16), tetanus toxin (17), and *Staphylococcus aureus* enterotoxin-A (18).

Despite these observations, however, neither the physiological role of $\gamma\delta$ T cells nor the cellular and molecular basis by which resting $\gamma\delta$ T cells are activated is understood. In this study we sought to determine if fresh resting $\gamma\delta$ T cells isolated from peripheral blood respond to allogeneic antigen-presenting cells and, if so, to assess the role in such responses of several cell surface molecules. The results indicate that dendritic cells (DC), but not monocytes, stimulate $\gamma\delta$ T cells to proliferate and that accessory molecules such as CD2, CD11a, and CD28 play critical roles in this process.

Methods

mAb. mAb directed against CD2 (Leu 5b), CD3 (Leu 4, OKT3), CD4 (Leu 3a, OKT4), CD5 (Leu 1), CD8 (Leu 2a, OKT8), CD11a (LFA-1 α), CD16 (Leu 11a, Leu 11c), CD19 (Leu 12), HLA-A,B,C (W6/32), HLA-DR (L243), and CD62L (Leu 8) were produced and purified in this laboratory. Anti- $\alpha\beta$ T cell receptor (TCR) (BMA031) and anti- $\gamma\delta$ TCR (TCR δ -1) mAb were purchased from T Cell Sciences, Inc., Cambridge, MA. mAb directed against CD56 (IOT56), CD72 (IOB72), CD1a (IOT6a), CD1b (IOT6b), and CD1c (IOT6c) were purchased from Amac, Inc., Westbrook, ME. Anti-CD28 (Leu 28) mAb was kindly provided by David Buck, Becton Dickinson & Co., San Jose, CA. Anti-CD80 (B7-1) mAb was a generous gift from Lewis L. Lanier, DNAX, Palo Alto, CA.

Cell separation. To isolate $\gamma\delta$ T and $\alpha\beta$, CD4+ T cells, PBMC obtained by Ficoll Hypaque (Sigma Chemical Co., St. Louis, MO) gradient centrifugation were separated into E-rosette positive (E+) and negative populations by a single step sheep erythrocyte rosetting method, using neuraminidase-treated sheep red blood cells. To obtain an enriched population of $\gamma\delta$ T cells, E+ cells were depleted of $\alpha\beta$ T cells and natural killer lymphocytes by incubating the cells with a mixture of mAb directed against CD4 (Leu 3), CD8 (Leu 2), and HLA-DR (L243) for 40 min at 4°C. After washing, the antibody-bound cells were removed by panning twice on plastic petri dishes coated with goat anti-mouse IgG as described (19). The enriched $\gamma\delta$ T cell fraction was further depleted of contaminating cells by a third and fourth panning procedure that used a mixture of anti- $\alpha\beta$ TCR, anti-CD56, and anti-CD16. To obtain CD28- $\gamma\delta$ T cells, purified $\gamma\delta$ cells were depleted of CD28+ cells by panning with anti-CD28 mAb. To enrich for $\alpha\beta$ TCR+, CD4+, CD8- T cells, E+ cells were depleted of contaminating cells by panning once with mAb directed against CD8, HLA-DR, and CD16.

Monocytes and DC were obtained from PBMC with modifications of a previously described method (20). 5×10^8 – 1×10^9 PBMC suspended in 7 ml of 5% human serum (HS) in Dulbecco's calcium- and magnesium-free PBS (DPBS) were overlaid onto a discontinuous Percoll (Pharmacia LKB, Uppsala, Sweden) gradient and centrifuged at 1,000 g for 25 min at 4°C. The gradient consisted of sequential layers of 75% (7 ml), 50.5% (16 ml), 40% (4 ml), and 30% (4 ml) solutions of stock isosmotic Percoll (1.129 g/ml, Pharmacia LKB) in 5% HS/DPBS. An enriched (70–85%) monocyte population was obtained at the interface between the 40 and 50.5% layers of Percoll (Pharmacia LKB). After three washes with 5% HS/DPBS, these cells were resuspended in RPMI 1640 medium supplemented with 2 mM L-glutamine,

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1. Abbreviations used in this paper. DC, dendritic cells; DPBS, Dulbecco's calcium- and magnesium-free phosphate buffered saline; E+, E-rosette positive; $\gamma\delta$ T cells, $\gamma\delta$ T cell receptor bearing lymphocytes; HS, human serum; TCR, T cell receptor.

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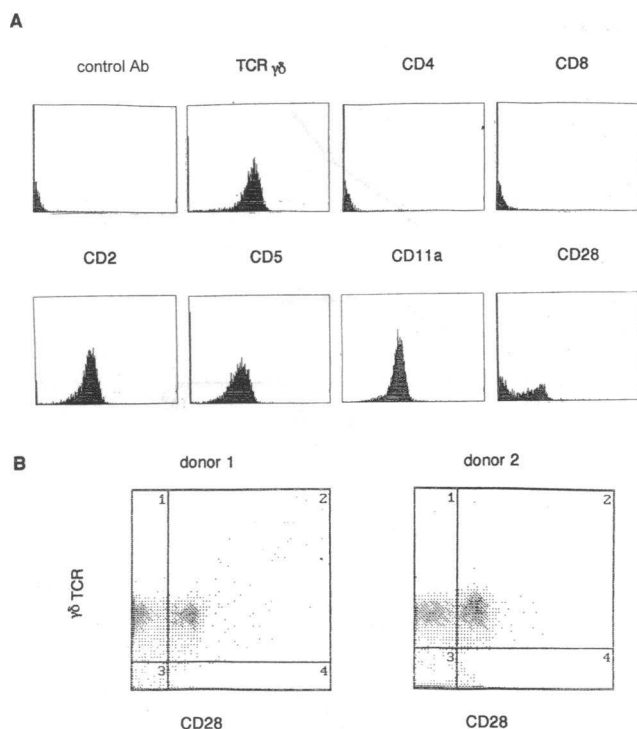


Figure 1. Cell surface antigens expressed by peripheral blood $\gamma\delta$ T cells. $\alpha\beta$ TCR $^-$, CD4 $^-$, CD8 $^-$, CD16 $^-$, CD56 $^-$ cells were enriched from E+ cells as described in Methods, and stained with FITC-conjugated mAb against $\gamma\delta$ TCR, CD4, CD8, CD2, CD5, CD11a, and CD28 (A). $\gamma\delta$ T cells obtained from two different donors were simultaneously stained with anti-CD28-FITC and anti- $\gamma\delta$ TCR-PE (B). 57% of donor 1 and 42% of donor 2 cells express CD28.

100 μ g/ml streptomycin, 100 U/ml penicillin, and 10% heat-inactivated pooled human serum (10% HS/RPMI) and incubated on glass petri dishes for 30 min in 10% CO₂ in air. The nonadherent cells were discarded and the adherent cells were collected by scraping.

The high density cell fraction from the Percoll (Pharmacia LKB) gradient was collected at the interface between the 50.5 and 75% layers and consisted of T cells, B cells, natural killer cells, and DC. The cells were suspended in 10% HS/RPMI and cultured overnight in Teflon vessels at 37°C in an atmosphere of 10% CO₂ in air. On the following day, the cells were suspended in 10 ml of 5% HS/DPBS and overlaid onto 10 ml of 15% (wt/vol) metrizamide in 10% HS/RPMI and centrifuged at 650 g for 10 min at room temperature (21). Cells in the low density layer (DC enriched) were resuspended in 10% HS/RPMI and incubated in Teflon vessels for 1–2 h. This population was further depleted of contaminating monocytes by a solid phase absorption (panning) procedure using human IgG-coated petri dishes (22). The unbound cells were then suspended in 2 ml of 5% HS/DPBS and layered over a second (14%) metrizamide gradient. To enrich DC to > 80% purity, a third (13%) metrizamide gradient was used.

Immunofluorescence analysis. Immunofluorescence analysis was performed as previously described (23). Expression of a variety of surface antigens on DC, monocytes, and T cells was determined on an Epics Profile II (Coulter Corp., Epics Division, Hialeh, FL) by single or two-color immunofluorescence. Antibodies were used as conjugates to FITC or PE or, if conjugates were not available, in combination with goat anti-mouse IgG-FITC.

Proliferation assays. 1×10^5 $\gamma\delta$ T cells or 5×10^4 $\alpha\beta$ T cells were cultured with 5×10^2 to 1×10^4 irradiated (3,000 rads) allogeneic DC or monocytes in U-bottomed microwells containing 200 μ l of 10% HS/RPMI for 6 d in an atmosphere of 10% CO₂ in air unless otherwise indicated. On the fifth day of culture 1 μ Ci of [³H]thymidine (6.7 Ci/mM; New England Nuclear, Boston, MA) was added to each well, and 16–20 h later the cells were harvested with a MASH apparatus (Otto Hiller Co., Madison, WI). The incorporation of [³H]thymidine into proliferating cells was determined with a liquid scintillation counter (Beckman Instruments, Inc., Palo Alto, CA). Results represent the mean

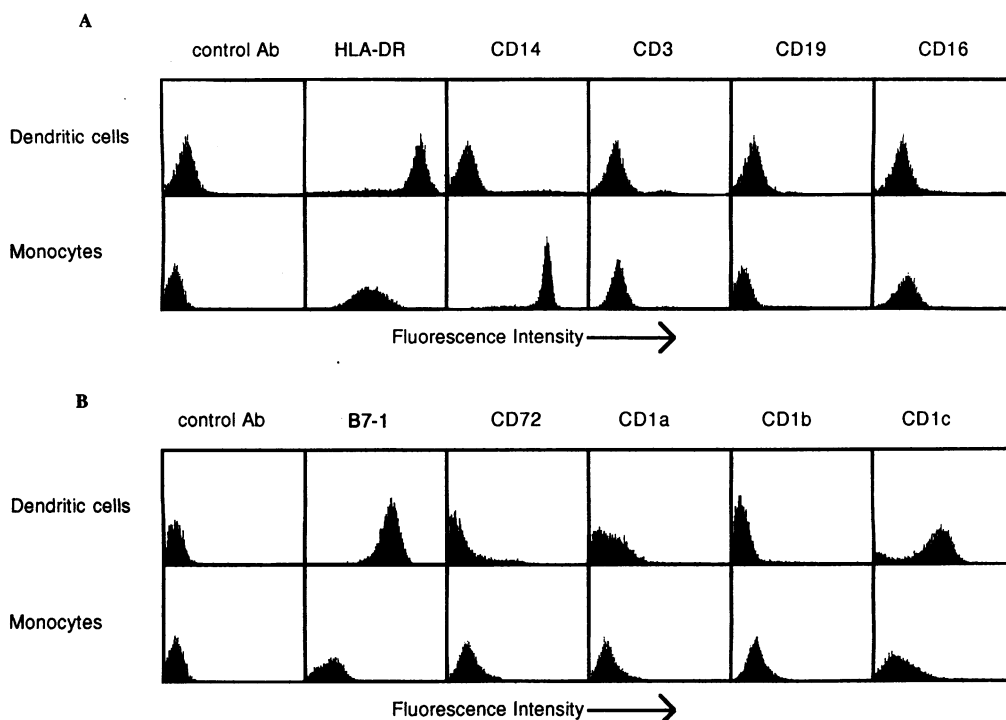


Figure 2. Cell-surface antigens expressed by DC and monocytes. DC and monocytes were purified from peripheral blood as described in Methods, and were stained with fluorochrome-conjugated mAb directed against HLA-DR, CD14, CD3, CD19, and CD16 (A), and by antibodies to CD80 (B7-1), CD72, CD1a, CD1b, and CD1c (B).

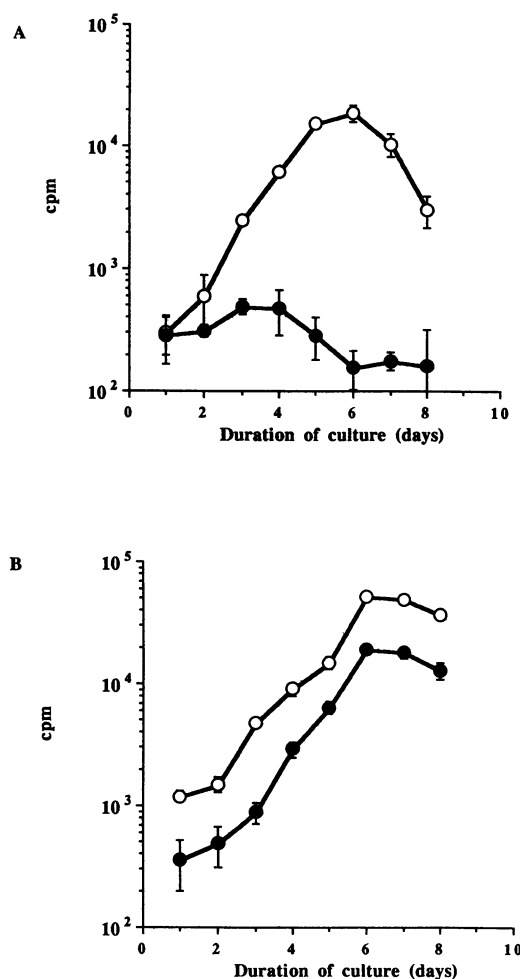


Figure 3. Ability of DC and monocytes to activate $\gamma\delta$ and $\alpha\beta$ T cells. (A) 1×10^5 $\gamma\delta$ T cells were cultured with 1×10^4 allogeneic DC (open circle) or monocytes (closed circle). (B) 5×10^4 $\alpha\beta$ T cells were cultured with 5×10^2 DC (open circle) or 5×10^3 monocytes (closed circle). The cells were harvested on the indicated days to determine [3 H]thymidine incorporation.

cpm \pm 1 SD of triplicate cultures. To investigate the kinetics of proliferation, the T cells were harvested 1–8 d after the initiation of culture, as described in the legend to Fig. 3. Where indicated (Table III) $\gamma\delta$ T cells were cultured in the presence of rIL2 and immobilized anti-CD3 mAb. For these studies 1×10^5 $\gamma\delta$ T cells were added to flat-bottomed microwells which had been incubated at 37°C overnight with 10 μ g/ml OKT3 and then washed. The cells were cultured for 5 d in these wells in the presence of 5 U/ml rIL2 (Cetus Corp., Berkeley, CA) under conditions otherwise identical to that described above.

To assess the effects of mAb to DC-associated surface molecules on the response of $\gamma\delta$ T cells, DC were incubated with the indicated mAb for 1 h at 4°C and then washed extensively. Thereafter, 6-d cultures were performed in the absence of added mAb, and the cultures were pulsed with [3 H]thymidine 16–20 h before harvesting. To assess the effects of anti-T cell mAb, T cells were incubated with the indicated mAb for 1 h at 4°C and, in addition, the 6-d cultures were performed in the presence of added mAb as indicated in the figure and table legends. Where indicated, mAb were added to the wells after varying periods of culture. Statistical analysis was performed by using the Student's *t* test.

Results

Immunofluorescence analysis of $\gamma\delta$ T cells and antigen-presenting cells. As described in detail in Methods, $\gamma\delta$ T cells were

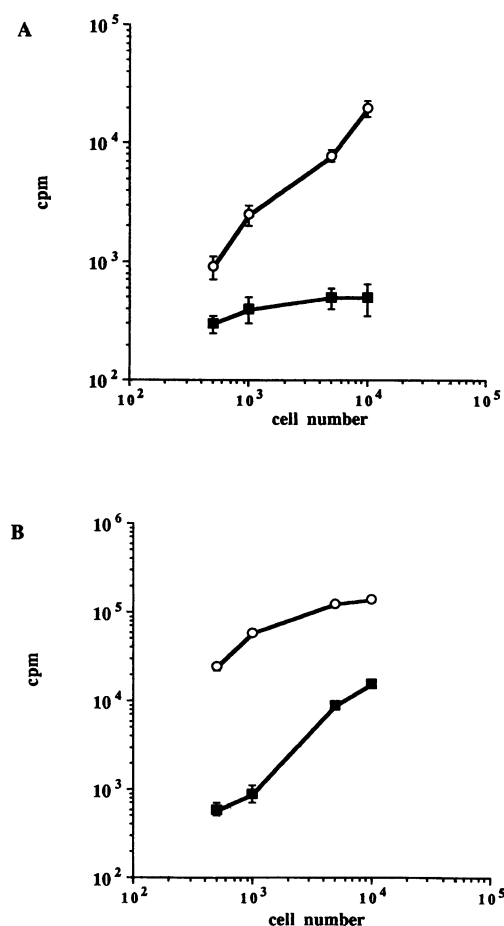


Figure 4. Effect of varying the number of allogeneic DC or monocytes on the proliferative response of $\gamma\delta$ T cells and $\alpha\beta$ T cells. 1×10^5 $\gamma\delta$ T cells (A) or 5×10^4 $\alpha\beta$ T cells (B) were cultured with graded numbers of DC (open circle) or monocytes (closed square) for 6 d. Cultures were pulsed with 1 μ Ci [3 H]thymidine 16–20 h before harvest. The results shown are representative of three experiments.

enriched from E+ cells by negative selection. More than 90% of these cells were $\gamma\delta$ TCR+, CD4–, CD8–, while < 1% were $\alpha\beta$ TCR+ (*n* = 7). The phenotype of the major contaminating cells was CD2+, CD3– (< 5%, data not shown). As shown in Fig. 1 A, nearly all of the purified $\gamma\delta$ T cells express CD2, CD5, and CD11a molecules, and 40–60% also expressed CD28 (*n* = 6) by two-color analysis as shown in Fig. 1 B.

Populations enriched for either DC or monocytes were separated from PBMC on the basis of their differential densities, as described in Methods. DC-enriched populations, prepared from more than 30 individuals, stained brightly with anti-HLA-DR mAb, but stained weakly or not at all for CD3, CD14, CD19, or CD16 (Fig. 2 A). In addition to HLA-DR, these cells also stained brightly with anti-CD80 (B7-1), anti-CD1c mAb, and HB-15 (not shown), but only weakly or not at all with mAb directed at CD1a or CD1b (Fig. 2 B). A small percentage of these cells stained with anti-CD72, consistent with slight B cell contamination. This cell population, which contained > 85% of cells with characteristic branched morphology, was used as DC for the assays described in this report. More than 95% of glass adherent cells obtained from the low density Percoll (Pharmacia LKB) fraction stained with both anti-CD14 and anti-HLA-DR mAb (Fig. 2 A). This monocyte-enriched frac-

Table 1. Effect of Antibodies to HLA and CD1 Antigens on the Response of T Cells to DC

mAb added	Response			
	Experiment 1		Experiment 2	
	$\gamma\delta$ T	$\alpha\beta$ T	$\gamma\delta$ T	$\alpha\beta$ T
	<i>cpm $\times 10^{-3}$</i>			
Responder alone	0.3	0.1	0.5	0.2
No antibody	17.3	36.8	12.7	46.6
Control				
(anti-CD62L)	17.4	38.6	12.1	48.6
Anti-HLA-DR	5.1 (71)*	18.2 (53)*	2.9 (76)*	21.3 (56)*
Anti-HLA-A,B,C	17.9	36.0 (7)	13.4	47.9 (2)
Anti-CD1a	18.5	37.3 (3)	11.7 (3)	45.0 (8)
Anti-CD1c	19.9	40.7	13.8	51.5

DC were pretreated with or without 5 μ g/ml anti-CD62L, anti-HLA-DR, anti-HLA-A,B,C, anti-CD1a, anti-CD1c for 1 h at 4°C and then washed extensively with cold medium before initiation of 6-d cultures with the indicated T cell population. Numbers in parentheses represent percent inhibition relative to control values. * Inhibition significant at $P < 0.01$. † Inhibition significant at $P < 0.05$.

tion was not stained by anti-B7-1, anti-CD1a, anti-CD1b, anti-CD1c mAb, or anti-CD72 (Fig. 2 B).

Ability of DC and monocytes to activate $\gamma\delta$ T cells. To determine whether fresh, resting $\gamma\delta$ T cells can recognize and respond to allogeneic antigen presenting cells, $\gamma\delta$ T cells were cultured with 1×10^4 purified DC or monocytes, and proliferation was measured on days 1–8 of culture. As shown in Fig. 3 A, $\gamma\delta$ T cells responded to DC with a peak response on days 5–6 of culture. In contrast, purified monocytes failed to activate $\gamma\delta$ T cells, regardless of the duration of culture (Fig. 3 A). $\alpha\beta$ T cells, on the other hand, responded well to both DC and monocyte stimulator populations (Fig. 3 B).

Response of $\gamma\delta$ and $\alpha\beta$ T cells to graded numbers of allogeneic DC or monocytes. As shown in Fig. 4 A, the response of $\gamma\delta$ T cells increased as a function of the number of DC added to the cultures. Although a plateau is not reached in the experiment shown, in other experiments we have observed that $2.5\text{--}5 \times 10^4$ DC yields the maximum response. The response of $\alpha\beta$ T cells to DC also increased in a dose-dependent manner (Fig. 4 B), and on a per cell basis the response of these cells exceeded that of $\gamma\delta$ T cells. $\alpha\beta$ T cells also responded, albeit less vigorously, to allogeneic monocytes, whereas $\gamma\delta$ T cells did not.

Effects of mAb to DC surface molecules on the response of

$\gamma\delta$ T cells. To explore the role of DC-associated surface molecules in the $\gamma\delta$ T cell response, DC were pretreated with mAb directed against HLA-DR, HLA class I, CD1a, or CD1c antigens, washed and then cultured with allogeneic $\gamma\delta$ T cells or $\alpha\beta$ T cells. Among these, mAb anti-HLA-DR mAb alone inhibited the proliferative responses of $\gamma\delta$ and $\alpha\beta$ T cells, whereas mAb against HLA class I and CD1 molecules had no effect (Table I).

Role of T cell surface molecules in the response of $\gamma\delta$ T cells to DC. To explore the role of $\gamma\delta$ T cell surface molecules in the response of these cells to allogeneic DC, cultures were performed in the presence of mAb directed at a variety of transmembrane molecules expressed on $\gamma\delta$ T cells, $\alpha\beta$ T cells or both. As shown in Fig. 5, anti-CD3 and anti- $\gamma\delta$ TCR mAb inhibited the response of $\gamma\delta$ T cells, whereas anti- $\alpha\beta$ TCR, anti-CD4, and anti-CD8 mAb had no effect. On the other hand, the response of $\alpha\beta$ CD4+ T cells was inhibited by anti-CD3, anti- $\alpha\beta$ TCR, and anti-CD4 mAb, but not by anti- $\gamma\delta$ TCR or anti-CD8 mAb. Table II shows that mAb to CD2, CD11, and CD28 inhibited the response of both $\gamma\delta$ and $\alpha\beta$ T cells. The results of three representative experiments demonstrated that anti-CD2 and anti-CD11a mAb inhibited the proliferative response of each T cell population to a similar extent, whereas anti-CD5 mAb had no effect on the response of either population. Although anti-CD28 mAb inhibited the response of both T cell populations, the inhibitory effect was substantially greater on $\gamma\delta$ T cells (72–85%) than on $\alpha\beta$ T cells (39–45%). As shown in Fig. 6, a mAb to B7-1 (CD80), which is a ligand of CD28, was less inhibitory of $\gamma\delta$ and $\alpha\beta$ T cell responses to DC than anti-CD28 mAb, but the effect of anti-B7-1 on the response of $\gamma\delta$ T cells was greater than the effect of this mAb on the $\alpha\beta$ T cell response. The inhibitory effect of anti-CD80 is seen even at low concentrations (0.01 μ g/ml) of antibody. We believe that this effect may be explained by the low number of DC (10^4) per well such that even a small amount of antibody is sufficient to bind most of the CD80 molecules and produce an inhibitory effect. The higher number of CD28+ $\gamma\delta$ T cells per well may explain why the inhibitory effect of anti-CD28 is reduced at low concentrations of antibody.

In light of the potent inhibitory effect of anti-CD28 on the response of $\gamma\delta$ T cells to allogeneic DC, further studies were undertaken to examine the basis of this effect. First, to determine when during the culture anti-CD28 exerted its effect, anti-CD28 mAb and a control mAb (CD62L) were added to cultures of $\gamma\delta$ cells and DC at 0, 24, 48, 72, and 96 h. The results (Fig. 7) show that the inhibitory effect of anti-CD28 mAb was seen during the first 24 h of culture and disappeared thereafter for both $\gamma\delta$ and $\alpha\beta$ T cells. Finally, since 40–60% of $\gamma\delta$ T cells

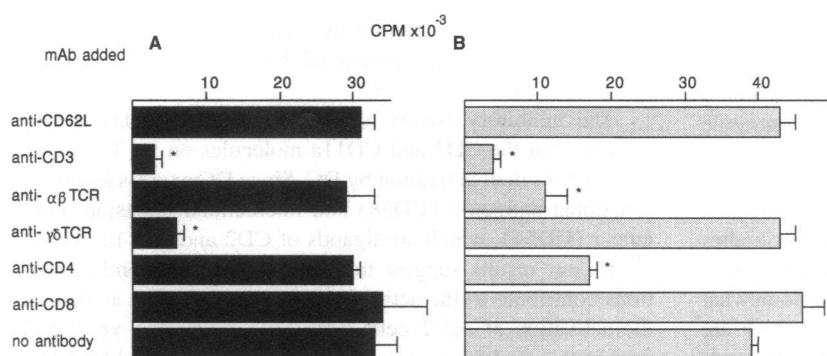


Figure 5. Effects of anti-CD3/TCR and anti-CD4/CD8 antibodies on the proliferative response of $\gamma\delta$ T cells (A) and $\alpha\beta$ T cells (B) to allogeneic DC. 1×10^5 $\gamma\delta$ T cells or 5×10^4 $\alpha\beta$ CD4+ T cells were cultured with 1×10^4 or 5×10^2 DC for 6 d in the presence of the indicated antibodies. The results shown are representative of five experiments. * Significant inhibition vs control ($P < 0.01$).

Table II. Effects of mAb against T Cell Surface Molecules on the Response of $\gamma\delta$ T Cells and $\alpha\beta$ T Cells to Allogeneic DC

mAb added	Response					
	Experiment 1		Experiment 2		Experiment 3	
	$\gamma\delta$ T	$\alpha\beta$ T	$\gamma\delta$ T	$\alpha\beta$ T	$\gamma\delta$ T	$\alpha\beta$ T
	<i>cpm</i> $\times 10^{-3}$					
Responder alone	0.3	0.1	0.5	0.1	0.3	0.2
No antibody	23.5	37.4	15.1	38.6	28.9	30.7
Control (anti-CD62L)	24.6	39.9	15.5	38.5	30.4	32.0
Anti-CD2	4.9 (80)*	14.0 (65)*	4.3 (72)*	14.2 (63)*	9.1 (70)*	10.0 (69)*
Anti-CD11a	5.1 (79)*	7.2 (82)*	2.5 (84)*	7.3 (81)*	8.5 (72)*	8.3 (74)*
Anti-CD28	6.9 (72)*	23.0 (42) [§]	3.1 (80)*	23.5 (39) [§]	4.6 (85)*	17.6 (45) [§]
Anti-CD5	25.8	38.4 (5)	17.6	35.1 (9)	33.0	33.9

1×10^5 $\gamma\delta$ T cells or 5×10^4 $\alpha\beta$ T cells from three different donors were cultured with 1×10^4 or 5×10^2 DC, respectively, for 6 d in the presence of the indicated antibodies (1 μ g/ml). Responder cells were preincubated with 1 μ g/ml of mAb for 1 h at 4°C. Numbers in parentheses represent percent inhibition relative to control values. * Inhibition significant at $P < 0.01$. [§] Inhibition significant at $P < 0.05$.

do not express CD28 (Fig. 1 B), we depleted $\gamma\delta$ T cells of CD28+ cells and evaluated the ability of the remaining CD28– cells to respond to allogeneic DC. Table III shows that the proliferative response of $\gamma\delta$ T cells was substantially diminished after depletion of CD28+ cells. Despite their inability to respond to alloantigen, however, the CD28 depleted $\gamma\delta$ T cells retained the ability to respond to immobilized anti-CD3 mAb in combination with IL-2.

Discussion

Although multiple $\gamma\delta$ T cell lines and clones have been described in published reports, the cellular and molecular basis by which freshly isolated, resting $\gamma\delta$ T cells are activated has not been addressed previously. The results of this study indicate that allogeneic DC, but not monocytes, induce $\gamma\delta$ TCR+, CD4–, CD8– T cells to proliferate in a dose-dependent manner. This result is not necessarily surprising in light of numerous studies of DC which have shown that this cell type is an extremely potent antigen-presenting cell for resting $\alpha\beta$ T cells. Indeed, in our study, $\alpha\beta$ CD4+ T cells responded even more strongly than $\gamma\delta$ T cells to DC. However, $\alpha\beta$ T cells also responded to monocytes, whereas $\gamma\delta$ T cells did not.

Critical to this study was the development of methods for isolating enriched populations of $\gamma\delta$ T cells, devoid of $\alpha\beta$ T cells, as well as methods for obtaining enriched populations of DC and monocytes. All cell types included in this study were obtained by negative selection to avoid the inhibitory effects of cell-bound antibodies while maintaining the ability to study the effects of antibodies added directly to the cultures. The response of $\gamma\delta$ T cells was inhibited by anti- $\gamma\delta$ TCR mAb, but not by anti- $\alpha\beta$ TCR, anti-CD4 or anti-CD8 mAb (Fig. 5), excluding the possibility that contaminating $\alpha\beta$ T cells might contribute to the response.

To investigate the role of DC-associated surface molecules in the activation of $\gamma\delta$ T cells, DC were incubated with selected mAb before their culture with T cells. Among the antibodies tested, anti-HLA-DR alone inhibited the proliferative response of $\gamma\delta$ T cells. Moreover, the inhibitory effect was somewhat stronger on $\gamma\delta$ T cells than on $\alpha\beta$, CD4+ T cells, which are known to recognize and respond to HLA-DR determinants and/

or complexes of DR and associated peptide (24). This result suggests that a proportion of circulating $\gamma\delta$ T cells capable of responding to allogeneic stimuli recognize HLA-DR and/or DR-associated molecules on DC. The recent finding that murine MHC class II restricted $\gamma\delta$ T cell hybridomas recognize unprocessed class II molecules indicates that the topology of MHC recognition differs between $\gamma\delta$ and $\alpha\beta$ TCRs and raises the possibility that the $\gamma\delta$ TCR is immunoglobulin-like in its antigen recognition (25). In our studies no attempt was made to define the fine specificity of the $\gamma\delta$ T cell proliferative response, and the inhibitory effect of anti-DR mAb is consistent with a role for either processed or unprocessed peptides in this response.

Although mAb to class I MHC as well as CD1a and c did not inhibit the allogeneic response of $\gamma\delta$ T cells, the possibility cannot be excluded that portions of these antigens which were not recognized by our mAb panel might play a role in this response. The genes in the human CD1 locus on chromosome 1 potentially encode five distinct nonpolymorphic proteins, which are designated CD1a, b, c, d, and e. CD1 proteins have been shown to be expressed on cells of dendritic morphology in a variety of tissues (26). In addition, two human $\gamma\delta$ T cell clones (V δ 1+) were found to kill CD1c-expressing target cells and the activity of these clones was inhibited by an anti-CD1c mAb (14, 15). CD1b determinants have also been reported to serve as restriction elements in the proliferative and cytotoxic response of CD4–CD8– $\alpha\beta$ T cells to microbial pathogens (27). The DC used in our studies expressed CD1a and CD1c, but not CD1b (Fig. 2 B). Nonetheless, antibodies specific for these proteins did not inhibit the allogeneic response of $\gamma\delta$ T cells, in contrast to the potent inhibitory effect of anti-HLA-DR (Table I).

The inhibitory effects of anti-CD2 and anti-CD11a mAb indicate that the CD2 and CD11a molecules on $\gamma\delta$ T cells are essential for their activation by DC. Since DC express leukocyte functional antigen-3 (CD58) and intercellular adhesion molecule-1 (CD54), which are ligands of CD2 and CD11a, respectively, our results suggest that these receptor–ligand interactions contribute to the activation of $\gamma\delta$ T cells, just as they do the activation of $\alpha\beta$ T cells (Table II) (28). The $\gamma\delta$ T cells used in this study also express CD5, which is a ligand for CD72

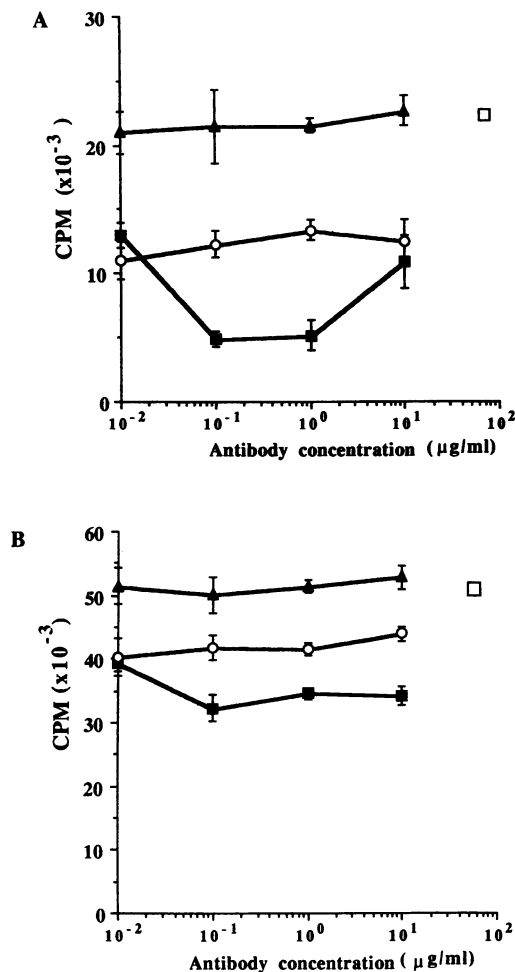


Figure 6. Effects of anti-CD28 and anti-CD80 antibodies on the proliferative response of $\gamma\delta$ T cells and $\alpha\beta$ T cells. 1×10^5 $\gamma\delta$ T cells (A) or 5×10^4 $\alpha\beta$ T cells (B) were cultured with 1×10^4 or 5×10^2 DC, respectively, for 6 d in the presence of the indicated concentrations of each mAb. The results shown are representative of three experiments. Statistical analysis for A: anti-CD80 vs control (anti-CD62L), $P < 0.05$; anti-CD28 vs control, $P < 0.01$; anti-CD80 vs anti-CD28, $P < 0.05$. B: anti-CD80 vs control, $P < 0.05$; anti-CD28 vs control, $P < 0.01$; anti-CD80 vs anti-CD28, $P < 0.05$. ■, anti-CD28; ○, anti-CD80; ▲, anti-CD62L; □, no antibody.

(29). In association with immobilized anti-CD3 mAb, anti-CD5 mAb enhance IL-2 receptor expression and IL-2 production and initiate T cell proliferation (30). However, while CD72 molecules are expressed by B cells, they are not expressed by most DC or monocytes (Fig. 2 B), and anti-CD5 mAb had no effect on the response of $\gamma\delta$ or $\alpha\beta$ T cells to DC (Table II). These results suggest that CD5 molecules do not play a role in allogeneic mixed leukocyte reaction, a result consistent with other studies (28).

CD28 molecules are expressed on all or nearly all CD4+ T cells, the majority of CD8+ T cells (31), and 40–60% of circulating $\gamma\delta$ T cells (Fig. 1 B) (32). The interaction between CD28 and the B7-1 (CD80) molecule, which is expressed on differentiated DC as well as activated B cells (33), γ -interferon-activated monocytes (34), and T cell clones (35), results in augmentation of $\alpha\beta$ T cell activation initiated through the TCR (36), apparently as a consequence of the induction of IL-2 transcription and IL-2 receptor expression (37, 38). In our

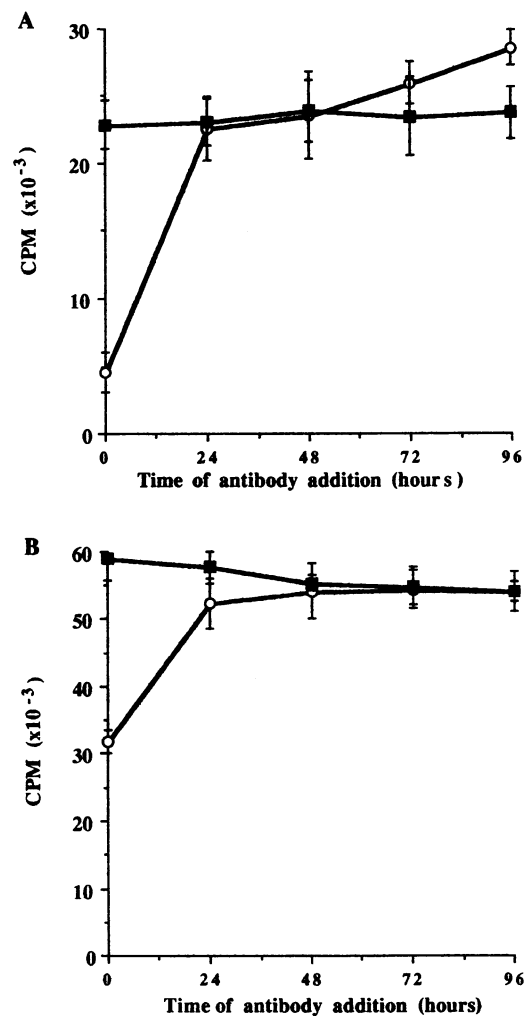


Figure 7. Time course of the effect of anti-CD28 mAb on the response of $\gamma\delta$ and $\alpha\beta$ T cells to DC. 1×10^5 $\gamma\delta$ T cells (A) or 5×10^4 $\alpha\beta$ T cells (B) were cultured with 1×10^4 or 5×10^2 DC, respectively, for 6 d. 0.1 $\mu\text{g/ml}$ anti-CD62L (control mAb) or anti-CD28 mAb was added to the cultures at the indicated times. The results shown are representative of four experiments. ○, CD28 mAb; ■, control mAb.

study anti-CD28 mAb inhibited the response of both $\gamma\delta$ and $\alpha\beta$ T cells in allogeneic mixed leukocyte reaction; however, the inhibitory effect was nearly twofold greater on $\gamma\delta$ T cells than on $\alpha\beta$ T cells (Table II, Fig. 6). Moreover, depletion of the CD28+ subset from purified $\gamma\delta$ T cells resulted in substantial reduction of the response to DC (Table III). These results suggest that the proliferative response of $\gamma\delta$ T cells induced by allogeneic DC may be more dependent upon the CD28 activation pathway than that of CD4+ T cells. It is well known that CD4 and CD8 molecules function to enhance the interaction between $\alpha\beta$ T cells and antigen presenting cells, thereby augmenting the response of these cells to antigenic stimuli (24). In addition, CD4 and CD8 molecules can independently transduce intracellular activation signals during antigenic stimulation (39, 40). We speculate that activation signals mediated through the TCR may be weaker in CD4–, CD8– $\gamma\delta$ T cells than in CD4+ or CD8+ $\alpha\beta$ T cells. This may explain the increased dependency of $\gamma\delta$ T cell activation on the CD28 costimulation pathway.

Like anti-CD28, the inhibitory effect of anti-B7-1 mAb was

Table III. Ability of CD28-depleted $\gamma\delta$ T Cells to Respond to DC

Donor	$\gamma\delta$ T cells	CD28- $\gamma\delta$ T cells
	Response to DC	
	cpm $\times 10^{-3}$	
1	12.5 \pm 2.6	2.1 \pm 0.3
2	9.6 \pm 1.6	1.5 \pm 0.2
3	18.7 \pm 2.5	2.4 \pm 0.2
Response to anti-CD3 + IL-2		
4	52.8 \pm 4.1	45.7 \pm 3.3
5	65.3 \pm 5.9	58.2 \pm 3.7

1×10^5 unfractionated $\gamma\delta$ T cells or CD28-depleted $\gamma\delta$ T cells were obtained from five donors and cultured with 1×10^4 allogeneic DC for 6 d (donors 1–3), or with anti-CD3 mAb and 5 U/ml rIL-2 for 5 d (donors 4 and 5). Results represent the mean cpm \pm 1 SD of triplicate cultures. [3 H]thymidine uptake of the responder T cells alone was < 400 cpm.

also twofold greater on $\gamma\delta$ than $\alpha\beta$ T cells (Fig. 6). However, the potency of inhibition was less than that seen with anti-CD28. This result may be explained by the existence of a second ligand, B7-2, for CD28 (41). Whereas B7-1 is expressed on activated but not resting antigen presenting cells, B7-2 is expressed on resting monocytes and DC as well as activated T, NK, and B cells. Anti-B7-2 mAb reportedly inhibits primary allogeneic mixed leukocyte reaction more efficiently than anti-B7-1, suggesting that the ligation of B7-2 and CD28 plays a significant role in the activation of resting $\alpha\beta$ T cells (41). Our results suggest that the interactions between CD28 and its ligands on DC play an important role in the activation of resting $\gamma\delta$ T cells. The observation that the inhibitory effect of anti-CD28 mAb occurs during the first 24 h of culture (Fig. 7) is consistent with this interpretation.

In the current study we have attempted to address the cellular and molecular basis of $\gamma\delta$ T cell activation, in contrast to previous studies, which have focused on the functions of activated $\gamma\delta$ cells. For example, a variety of $\gamma\delta$ T cell lines and clones with cytotoxic activity have been generated from alloantigen stimulated cultures (9, 11, 42–44), and in some examples the cytolytic activity has been restricted by MHC class I (9, 11) or II (44) determinants. Since noncytolytic, immunoregulatory effects have also been suggested to be mediated by $\gamma\delta$ T cells (45, 46), $\gamma\delta$ T cells activated by allogeneic DC may be functionally heterogeneous. Regardless of their precise function(s), however, it is apparent that the cell-surface molecules described in this report are critical to the generation of effector cells that mediate these functions.

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References

- Groh, V., S. Porcelli, M. Fabbri, L. L. Lanier, L. J. Picker, T. Anderson, R. A. Warnke, A. K. Bhan, J. L. Strominger, and M. B. Brenner. 1989. Human lymphocytes bearing T cell receptor $\gamma\delta$ are phenotypically diverse and evenly distributed throughout the lymphoid system. *J. Exp. Med.* 169:1277–1294.

- Bucy, R. P., C.-L. H. Chen, and M. D. Cooper. 1989. Tissue localization and CD8 accessory molecule expression of T $\gamma\delta$ cells in humans. *J. Immunol.* 142:3045–3049.
- Modlin, R. L., C. Pirmez, F. M. Hofman, V. Torigan, K. Uyemura, T. H. Rea, B. R. Bloom, and M. B. Brenner. 1989. Lymphocytes bearing antigen-specific $\gamma\delta$ T cell receptors accumulate in human infectious disease lesions. *Nature (Lond.)* 338:554–548.
- Russo, D. M., R. J. Armitage, M. Barral-Netto, A. Barral, K. H. Grabstein, and S. G. Reed. 1993. Antigen-reactive $\gamma\delta$ T cells in human Leishmaniasis. *J. Immunol.* 151:3712–3718.
- Roussilhon, C., M. Agrapart, J.-J. Ballet, and A. Bensussan. 1990. T lymphocytes bearing the $\gamma\delta$ T cell receptor in patients with acute plasmodium falciparum malaria. *J. Infect. Dis.* 162:283–285.
- DePaoli, P., D. Gennari, P. Martelli, V. Cavazzerani, R. Comoretto, and G. Santini. 1990. $\gamma\delta$ T-cell receptor bearing lymphocytes during Epstein-Barr Virus infection. *J. Infect. Dis.* 161:1013–1016.
- Autran, B., F. Triebel, C. Katlama, W. Rosenbaum, T. Hercend, and P. Debre. 1989. T-cell receptor $\gamma\delta$ lymphocyte subsets during HIV infection. *Clin. Exp. Immunol.* 75:206–210.
- DePaoli, P., D. Gennari, P. Martelli, G. Basaglia, M. Grovatto, and S. Battistin. 1991. A subset of lymphocytes is increased during HIV-1 infection. *Clin. Exp. Immunol.* 83:187–191.
- Ciccone, E., O. Viale, D. Pende, M. Malnati, G. B. Ferrara, S. Barocci, A. Moretta, and L. Moretta. 1989. Specificity of human lymphocytes expressing a $\gamma\delta$ T cell antigen receptor. Recognition of a polymorphic determinant of HLA class I molecules by a $\gamma\delta$ T cell clone. *Eur. J. Immunol.* 19:1267–1271.
- Rivas, A., J. Koide, M. L. Cleary, and E. G. Engleman. 1989. Evidence for involvement of the $\gamma\delta$ T cell antigen receptor in cytotoxicity mediated by human alloantigen-specific T cell clones. *J. Immunol.* 142:1840–1846.
- Spits, H., X. Paliard, V. H. Engelhard, and J. E. deVries. 1990. Cytotoxic activity and lymphokine production of T cell receptor (TCR) $-\alpha\beta$ and TCR $-\gamma\delta$ cytotoxic T lymphocyte (CTL) clones recognizing HLA-A2 and HLA-A2 mutants. *J. Immunol.* 144:4156–4162.
- Vandekerckhove, B. A. E., G. Datema, F. Koning, E. Goulmy, G. G. Persijn, J. J. Van Rood, F. H. J. Claas, and J. E. De Vries. 1990. Analysis of the donor-specific cytotoxic T lymphocyte repertoire in a patient with a long-term surviving allograft. *J. Immunol.* 144:1288–1294.
- Jitsukawa, S., F. Triebel, F. Faure, C. Miossec, and T. Hercend. 1988. Cloned CD3+ TcR α/β –TiaA– peripheral blood lymphocytes compared to the TiaA+ counterparts: structural differences of the $\gamma\delta$ receptor and functional heterogeneity. *Eur. J. Immunol.* 18:1671–1679.
- Porcelli, S., M. B. Brenner, J. L. Greenstein, S. P. Balk, C. Terhorst, and P. A. Bleicher. 1989. Recognition of cluster of differentiation 1 antigens by human CD4–CD8– cytolytic T lymphocytes. *Nature (Lond.)* 341:447–450.
- Faure, F., S. Jitsukawa, C. Miossec, and T. Hercend. 1990. CD1c as a target recognition structure for human T lymphocytes: analysis with peripheral blood $\gamma\delta$ cells. *Eur. J. Immunol.* 20:703–706.
- Holoshitz, J., F. Konig, J. E. Coligan, J. De Bruyn, and S. Strober. 1989. Isolation of CD4–CD8– mycobacteria-reactive T lymphocyte clones from rheumatoid arthritis synovial fluid. *Nature (Lond.)* 339:226–229.
- Holoshitz, J., L. M. Vial, B. J. Keraock, D. R. McKinley, and N. K. Bayne. 1992. Dual antigenic recognition of cloned human $\gamma\delta$ T-cells. *J. Clin. Invest.* 89:308–314.
- Rust, C. J. J., F. Verreck, H. Vietor, and F. Koning. 1990. Specific recognition of staphylococcal enterotoxin A by human T cells bearing receptors with the V γ 9 region. *Nature (Lond.)* 346:572–574.
- Engleman, E. G., C. J. Benike, F. C. Grumet, and R. L. Evans. 1981. Activation of human T lymphocyte subsets: helper and suppressor/cytotoxic cells recognize and respond to distinct histocompatibility antigens. *J. Immunol.* 127:2124–2129.
- Markowicz, S., and E. G. Engleman. 1990. Granulocyte-macrophage colony-stimulating factor promotes differentiation and survival of human peripheral blood dendritic cells in vitro. *J. Clin. Invest.* 85:955–961.
- Freudenthal, P. S., and R. M. Steinman. 1990. The distinct surface of human dendritic cells, as observed after an improved isolation method. *Proc. Natl. Acad. Sci. USA* 87:7698–7702.
- Young, J. W., and R. M. Steinman. 1988. Accessory cell requirements for the mixed leukocyte reaction and polyclonal mitogens, as studied with a new technique for enriching blood dendritic cells. *Cell. Immunol.* 111:167–182.
- Sasaki, D. T., S. E. Dumas, and E. G. Engleman. 1987. Discrimination of viable and non-viable cells using propidium iodide in two color immunofluorescence. *Cytometry* 8:413–420.
- Parnes, J. R. 1989. Molecular biology and function of CD4 and CD8. *Adv. Immunol.* 44:265–311.
- Schild, H., N. Mavaddat, C. Litzenberger, E. W. Ehrlich, M. M. Davis, J. A. Bluestone, L. Matis, R. K. Draper, and Y.-H. Chin. 1994. The nature of major histocompatibility complex recognition by $\gamma\delta$ T cells. *Cell* 76:29–37.
- Porcelli, S., M. B. Brenner, and H. Band. 1991. Biology of human $\gamma\delta$ T-cell receptor. *Immunol. Rev.* 120:137–183.
- Porcelli, S., C. T. Morita, and M. B. Brenner. 1992. CD1b restricts the

response of human CD4-CD8- T lymphocytes to a microbial antigen. *Nature (Lond.)*. 360:593-597.

28. Young, J. W., L. Koulouva, S. A. Soergel, E. A. Clark, R. M. Steinman, and B. Dupont. 1992. The B7/BB1 antigen provides one of several costimulatory signals for the activation of CD4+ T lymphocytes by human blood dendritic cells in vitro. *J. Clin. Invest.* 90:229-237.

29. Van de Velde, H., I. von Hoegen, W. Luo, J. R. Parnes, and K. Thielemans. 1991. The B-cell surface protein CD72/Lyb-2 is the ligand for CD5. *Nature (Lond.)*. 351:662-665.

30. Ceuppens, J. L., and M. L. Baroja. 1986. Monoclonal antibodies to the CD5 antigen can provide the necessary second signal for activation of isolated resting T cells by solid-phase-bound OKT3. *J. Immunol.* 137:1816-1821.

31. Linsley, P. S., and J. A. Ledbetter. 1993. The role of the CD28 receptor during T cell responses to antigen. *Annu. Rev. Immunol.* 11:191-212.

32. Testi, R., and L. L. Lanier. 1989. Functional expression of CD28 on T cell antigen receptor γ/δ -bearing T lymphocytes. *Eur. J. Immunol.* 19:185-188.

33. Freeman, G. J., A. S. Freedman, J. M. Segil, G. Lee, J. F. Whitman, and L. M. Nadler. 1989. B7, a new member of the Ig superfamily with unique expression on activated and neoplastic B cells. *J. Immunol.* 143:2714-2722.

34. Freedman, A. S., G. J. Freeman, K. Rhynhart, and L. M. Nadler. 1991. Selective induction of B7/BB-1 on interferon- γ stimulated monocytes: a potential mechanism for amplification of T cell activation through the CD28 pathway. *Cell. Immunol.* 137:429-437.

35. Azuma, M., H. Yssel, J. H. Phillips, H. Spits, and L. L. Lanier. 1993. Functional expression of B7/BB1 on activated T lymphocytes. *J. Exp. Med.* 177:845-850.

36. Linsley, P. S., W. Brady, L. Grosmaire, A. Aruffo, N. K. Damle, and J. A. Ledbetter. 1991. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. *J. Exp. Med.* 173:721-730.

37. Lindsten, T., C. H. June, J. A. Ledbetter, G. Stella, and C. B. Thompson. 1989. Regulation of lymphokine messenger RNA stability by a surface-mediated T cell activation pathway. *Science (Wash. DC)*. 244:339-343.

38. Fraser, J. D., B. A. Irving, G. R. Crabtree, and A. Weiss. 1991. Regulation of interleukin-2 gene enhancer activity by the T cell accessory molecule CD28. *Science (Wash. DC)*. 251:313-316.

39. Veillette, A., A. Bookman, E. M. Horak, L. E. Samelson, and J. B. Bolen. 1989. Signal transduction through the CD4 receptor involves the activation of the internal membrane protein tyrosine kinase p56^{lck}. *Nature (Lond.)*. 338:257-259.

40. Miceli, M. C., and J. R. Parnes. 1991. The roles of CD4 and CD28 in T cell activation. *Semin. Immunol.* 3:133-141.

41. Azuma, M., D. Ito, H. Yagita, K. Okumura, J. H. Phillips, L. L. Lanier, and C. Somoza. 1993. B70 antigen is a second ligand for CTLA-4 and CD28. *Nature (Lond.)*. 336:76-79.

42. Kabelitz, D., A. Bender, S. Schondelmaier, M. L. Da Silva Lobo, and O. Janssen. 1990. Human cytotoxic lymphocytes. V. Frequency and specificity of $\gamma\delta$ + cytotoxic lymphocyte precursors activated by allogeneic or autologous stimulator cells. *J. Immunol.* 144:2827-2832.

43. Haas, W., P. Pereira, and S. Tonegawa. 1993. Gamma/delta cells. *Annu. Rev. Immunol.* 11:637-685.

44. Matis, L. A., A. M. Fry, R. Q. Cron, M. M. Cotterman, R. F. Dick, and J. A. Bluestone. 1989. Structure and specificity of a class II MHC alloreactive $\gamma\delta$ T cell receptor heterodimer. *Science (Wash. DC)*. 245:746-49.

45. Mombaerts, P., J. Arnoldi, F. Russ, S. Tonegawa, and S. H. E. Kaufmann. 1993. Different roles of $\alpha\beta$ and $\gamma\delta$ T cells in immunity against an intracellular bacteria pathogen. *Nature (Lond.)*. 365:53-56.

46. Kaufmann, S. H. E., C. Blum, and S. Yamamoto. 1993. Crosstalk between α/β T cells and γ/δ T cells in vivo: activation of α/β T-cell responses after γ/δ T-cell modulation with the monoclonal antibody GL3. *Proc. Natl. Acad. Sci. USA*. 90:9620-9624.