

DIRECT ACTIVATION OF CD8⁺ CYTOTOXIC T LYMPHOCYTES BY DENDRITIC CELLS

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The basis for specificity during many cell-mediated immune responses has been substantially clarified. T lymphocytes express individualized or clonotypic antigen-recognition molecules, termed Ti, which recognize epitopes presented by class I and II products of the MHC (1–3). Polymorphic determinants on MHC products seem to serve directly as antigens in transplantation. MHC molecules also act as restriction elements to present other foreign antigens such as infectious agents and soluble proteins (4).

In addition to specificity, a notable aspect of immune responsiveness is that antigen be administered on a specialized accessory population termed dendritic cells (reviewed in reference 5). One response that is initiated by dendritic cells is the development of CTL (6, 7). Most CTL have the phenotype CD4⁻,CD8⁺. Early studies (8–10) described the amplifying role of Th cells (having the CD4⁺CD8⁻ phenotype) in the generation of CD8⁺ CTL. The helper cells would release lymphokines, particularly IL-2, which seemed essential for CTL development. Recent studies (11–13) have indicated that CD8⁺ T cells can be stimulated directly to become CTL and produce IL-2, i.e., in the absence of CD4⁺ T cells. We have studied the cellular requirements for the direct activation of CD8⁺ CTL. We report that dendritic cells are active accessory cells for this pathway. Macrophages, which bear readily detectable class I alloantigen, do not initiate an MLR from the CD8⁺ subset but serve as targets for the CTL that are induced by dendritic cells. The initial response of isolated CD8⁺ lymphocytes to dendritic cells is comparable in many respects to CD4⁺ cells, including the formation of dendritic-T cell clusters and the release of IL-2.

Materials and Methods

Mice. BALB/c × DBA/2 (CxD2; H-2 dxd)F₁, C57BL/6 × DBA/2 (B6D2; H-2 bxd)F₁, B6.H-2k, and C57BL/6 mice of both sexes, 6–12 wk old (The Trudeau Institute, Saranac Lake, NY), were used with similar results.

Cells. Enriched populations of dendritic cells were isolated as a low-density, macrophage-depleted, spleen-adherent fraction as described (14). These fractions were >90% irregularly shaped, Fc receptor-negative, Ia-rich, 33D1 antigen-positive, dendritic cells (7, 15).

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T lymphocytes were nylon-wool nonadherent spleen and lymph node suspensions that were treated with anti-Ia mAb (clones 10-2.16, anti I-A^k; 14-4-5S anti I-E^{d,k}; or B21-2 anti-Ia^{b,d}; American Type Culture Collection [ATCC], Bethesda, MD) along with an mAb to a T cell subset. The cells were treated at a final concentration of 10⁷ leukocytes/ml and 5.5% fresh rabbit serum (Pel-Freeze Biologicals, Rodgers, WI). The cytotoxic anti-T cell antibodies were GK 1.5 (rat anti-mouse L3T4 or anti-CD4; reference 16), H0 2.2 (mouse anti-Lyt-2 or anti-CD8; reference 17), C3PO (mouse anti-Lyt-1 or anti-CD5; reference 18). Rat mAb, also obtained from the ATCC, were used to monitor cell surface antigens by indirect immunofluorescence. The mAb included 53-6.7 anti-CD8, 53-7.3 anti-CD5, GK 1.5 anti-CD4, F448.1 anti-LFA-1, M1/70 anti-C3bi receptor, and 7D4 anti-IL-2-R. Cells were affixed to multiwell slides (Carlson Scientific, Peotone, IL) that had been coated with 75 µg/ml poly-L-lysine in PBS and stained successively with hybridoma culture supernatants followed by FITC mouse anti-rat Ig (No. 605-540; 2 µg/ml; Boehringer Mannheim Biochemicals, Indianapolis, IN). The CD8⁻ and CD4⁻ T cell subsets were at least 80–90% CD4⁺ or CD8⁺ with <1% contamination by the subset killed by the mAb. The purity of the responding subset was further verified by functional experiments in which we found that the proliferating cells in the MLR were CD8⁺, CD4⁻ and that the response was not inhibited by anti-CD4 mAb (reference 12; see Results).

Peritoneal macrophages were obtained by lavage of normal or thioglycollate-elicited mice. 1-d plastic adherent (>95% macrophages by cytology and expression of the F4/80 and M1/70 antigens; reference 19) resident and elicited macrophages were used as stimulators with similar results. Peritoneal macrophages were also used as ⁵¹Cr targets for CTL assays as described (20). Briefly, macrophages from stimulator (allogeneic) or responder (syngeneic) mouse strains were labeled with Na⁵¹CrO₄ either fresh or after culture in Teflon beakers. 5,000 macrophages were plated in triplicate in round-bottomed microtest wells. Graded doses of washed effector cells from the MLR were added for 4 h, and the supernatants were measured for ⁵¹Cr-release. Spontaneous release was <20% of total release in the presence of 0.1% SDS. Spleen adherent cells, which contain both macrophages and dendritic cells, were prepared from total or low-density spleen populations (14) and cultured overnight. Macrophages were enriched by eliminating most dendritic cells by treatment with 33D1 mAb and complement (7, 15).

Mixed Leukocyte Reactions. Graded doses of irradiated (900 rad ¹³⁷Cs) dendritic cells or other leukocytes (see Results) were added to syngeneic and allogeneic T cell subsets in flat-bottomed, microtest (6 mm) or macrotest (16 mm) wells. Microcultures contained 0.2 ml medium and 3 × 10⁵ T cells, and macrocultures had 1.0 ml medium and 3 × 10⁶ T cells. The medium was RPMI 1640 supplemented with 5% FCS, 20 µg/ml gentamicin sulfate, and 5 × 10⁻⁵ M 2-ME.

Dendritic cells-T cell clusters were isolated from the MLR by velocity sedimentation as described (21). The frequency of dendritic cells in the clusters was determined by using dendritic cells that had been tagged with a nontoxic carbocyanine dye as described (22). Isolated dendritic-T cell clusters were dissociated by pipetting before counting total and dye-labeled cells in a hemocytometer.

Proliferation in the MLR was measured by adding [³H]TdR at 4 µCi/ml for 12–16 h at the indicated times. CTL were assayed on ⁵¹Cr-labeled macrophages as targets (see above). T cell growth factor was assayed by adding graded doses of culture medium to 10⁴ Con A-induced T blasts in a total volume of 100 µl medium and by measuring [³H]-TdR uptake at 18–24 h (23). Growth factor activity was neutralized >80% by the S4B6 anti-IL-2 mAb, kindly provided by Dr. T. Mossman (24) and was used as a culture supernatant at 10% vol/vol. S4B6 did not block murine rIL-4 (provided by Dr. T. Mossman, DNAX, Palo Alto, CA) or human IL-2 (provided by Dr. S. Rudnick, Biogen, Cambridge, MA).

Results

Comparison of the CD4⁺ and CD8⁺ Responses to Allogeneic Dendritic Cells. Both CD4⁺ and CD8⁺ T cell subsets proliferated vigorously when challenged with

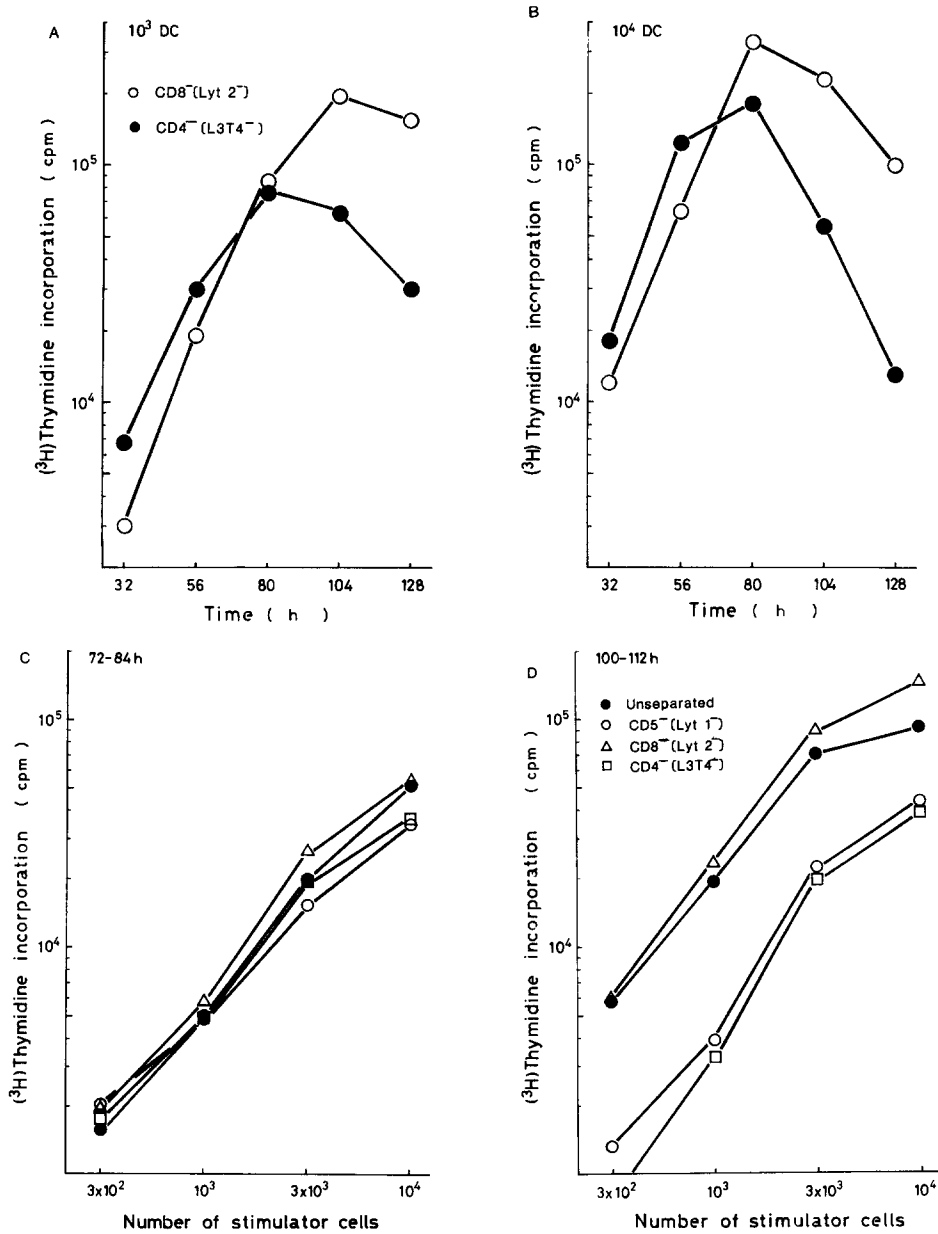


FIGURE 1. CD4⁺ (Lyt-2⁻) and CD8⁺ (L3T4⁻) T cell responses to enriched dendritic cells. (A and B) The kinetics of the MLR as illustrated with 10³ (A) and 10⁴ (B) dendritic cells from BALB/c × DBA/2 (CxD2; H-2d) mice stimulating T cell subsets from B6.H-2k mice. (C and D) Dose-response curves illustrated with dendritic cells from C57BL/6 × DBA/2 (B6xD2; H-2bd) mice stimulating CxD2 T cells for 4 (A) or 5 (B) d.

allogeneic dendritic cells in the three strain combinations that were tested (Fig. 1). Only 10³–10⁴ dendritic cells were needed to induce a strong MLR in 3 × 10⁵ CD8⁺ cells. CD8⁺ cells ceased responding at earlier time points than CD4⁺ cells

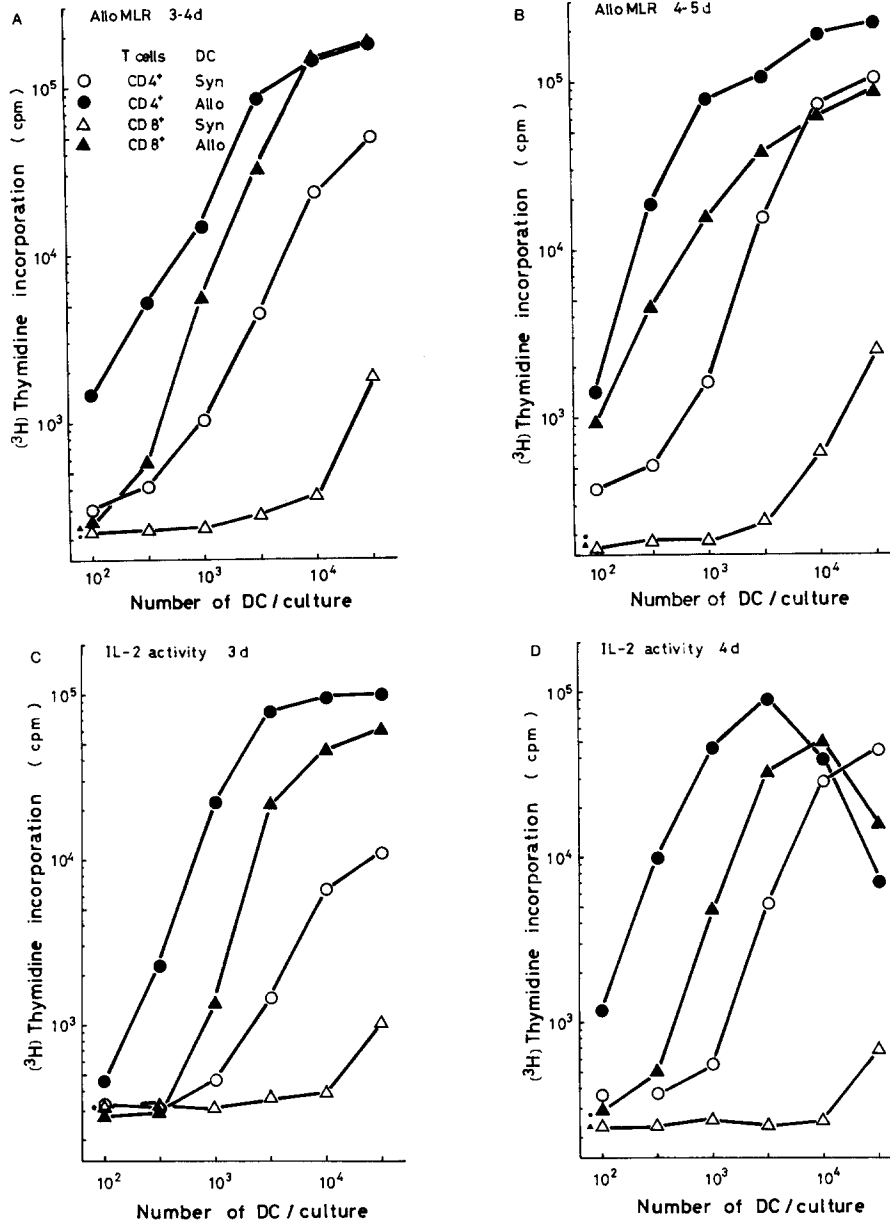


FIGURE 2. Proliferation and IL-2 production during the response of CD4⁺ and CD8⁺ subsets to syngeneic and allogeneic stimulators. (A and B) Comparison of the proliferative response to dendritic cells from B6.H-2k mice and T cells from syngeneic B6.H-2k or allogeneic Cx2D2 mice, at day 4 (A) and 5 (B). (C and D) IL-2 in the medium of cultures from Fig. 2, A and B. Growth factor activity in the dendritic-T cell-conditioned medium was assayed (50% vol/vol) on 3 × 10⁴ Con A blasts. Maximum values in the bioassay were 10⁵ cpm. The growth factor was likely to be IL-2 (see text).

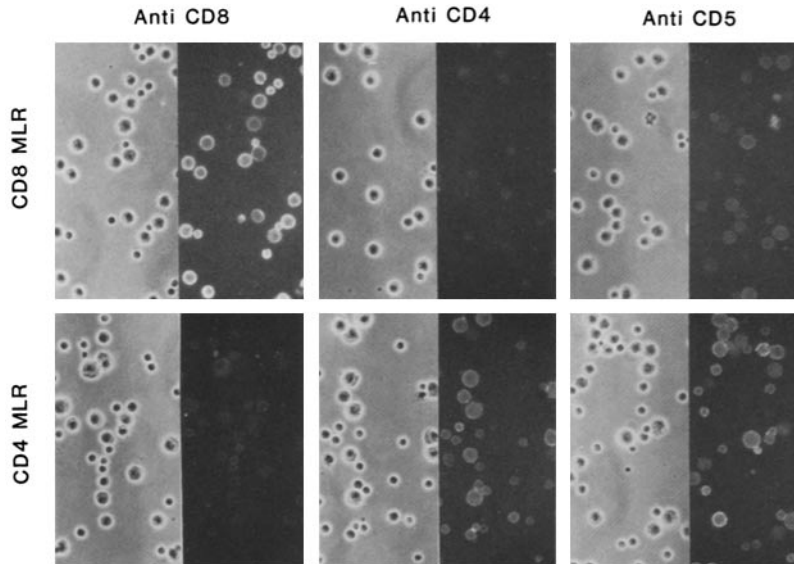


FIGURE 3. Cell-surface markers of lymphocytes in the CD4⁺ and CD8⁺ MLRs. Leukocytes were harvested at day 4 and applied to multiwell slides. The cells were exposed to mAb (anti-CD4 or L3T4, GK1.5; anti-CD8 or Lyt-2, 53-6.7; and anti-CD5 or Lyt-1, 53-7.1) followed by FITC-mouse anti-rat Ig. Phase-contrast and immunofluorescence micrographs ($\times 300$) are shown to indicate that most cells, including the lymphoblasts, in the CD8⁺ MLR were CD4⁻, CD8⁺ and vice versa for the CD4⁺ MLR. Not shown are stains with a panel of additional mAb which indicated that the large lymphoblasts in both MLRs were positive for Thy-1, LFA-1, and IL-2-R antigens but negative for macrophage and Fc receptor antigens.

(Fig. 1) and did not exhibit a syngeneic MLR (Fig. 2), as noted previously (12). T cell growth factor activity was readily detected when allogeneic dendritic cells stimulated CD8⁺ lymphocytes (Fig. 2, C and D). This growth factor was likely to be IL-2, since its activity was totally blocked by an mAb S4B6 (24) that neutralized IL-2 but not IL-4 (data not shown).

Evidence that Residual CD4⁺ T Cells Are Not Required for the CD8⁺ Response. When cells from the CD8⁺ MLR were examined by indirect immunofluorescence, most of the T cells, including the lymphoblasts, stained brightly with anti-CD8 mAb and FITC-mouse anti-rat Ig, but not at all with anti-CD4 mAb (Fig. 3). Many CD8⁺ lymphoblasts stained weakly with anti-CD5 (Lyt-1) mAb as well (Fig. 3).

Functional criteria were used to rule out a contribution by low but significant numbers of residual CD4⁺ cells. It is known that anti-CD4 or anti-Ia antibodies block CD4⁺ but not CD8⁺ responses (12, 25). This proved to be the case in our cultures (Fig. 4). Anti-CD4 (anti-L3T4) and anti-Ia antibodies only blocked the CD4⁺ MLR, while anti-CD8 (anti-Lyt-2) only blocked the CD8⁺ response. Both T cell subsets were inhibited by anti-LFA-1 antibodies, but not by a large panel of mAb to other dendritic and T cell surface antigens (Fig. 4, legend).

Development and Assay of Cytolytic T Cells. In addition to proliferative responses, allospecific lytic activity was evident in CD8⁺ MLRs generated in the presence of anti-CD4 mAb (Table I). We conclude that dendritic cells can directly stimulate the CD8⁺ subset to proliferate and differentiate.

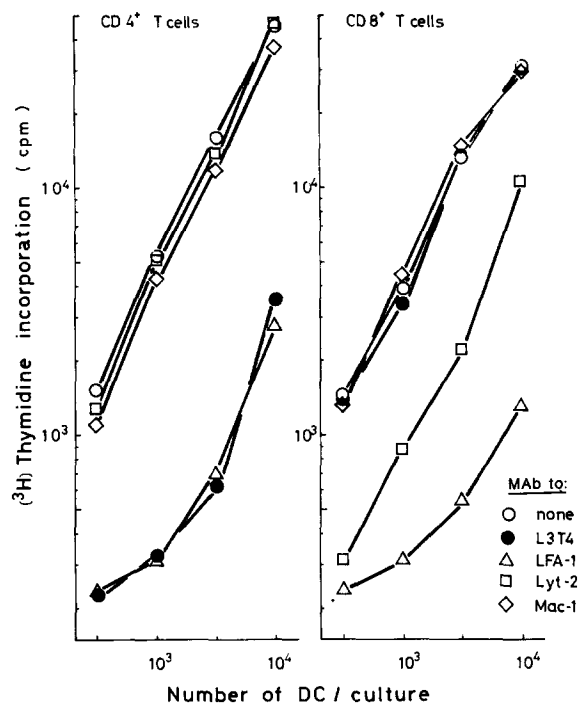


FIGURE 4. Effects of mAb on the CD4⁺ and CD8⁺ MLRs. Dendritic cells from B6.H-2k mice were added to 3×10^5 CD4⁺ or CD8⁺ T cells from CxD2 mice in the presence of various mAb that were obtained from the American Type Culture Collection and directed to the determinants listed in the legend. The mAb were GK 1.5, anti-CD4 or anti-L3T4; 53-6.7, anti-CD8 or anti-Lyt-2; FD441.8, anti-CD11b or anti-LFA-1; and a mixture of anti-I-A and I-E mAb, 10-2.16, and 14-4-5S. Hybridoma culture supernatants were added at 25% vol/vol, which was two to five times the dose needed for optimal blocking (not shown). Several isotype-matched mAb were also tested but produced no blocking (not shown), including: 53-7.3, anti-CD5 or Lyt-1; B5-3, anti-Thy-1; 33D1, anti-DC; M1/70, anti-C3biR; M1/42, anti-H-2K; M1/9.3, anti-leukocyte. Similar findings were made in four experiments.

Relative Efficacy of Dendritic Cells and Other Leukocytes as Stimulators. Other leukocyte populations were tested as accessory cells for the initiation of the CD8⁺ MLR. The first approach was to selectively reduce the trace dendritic cell component in heterogeneous populations. Spleen cells (~60% B lymphocytes, 3% macrophages, and 0.3% dendritic cells) and spleen adherent cells (~80% macrophages and 10% dendritic cells) were treated with the mAb 33D1 and complement (7, 15). This mAb does not block dendritic cell function; but in the presence of complement, 33D1 selectively kills dendritic cells and eliminates function in several primary responses (7, 26, 27). Selective lysis of dendritic cells eliminated 80–95% of the stimulating activity for both CD4⁺ or CD8⁺ MLRs (Fig. 5).

Whole spleen populations consistently were at least 10 times less effective in stimulating the CD8⁺ subset as the CD4⁺ subset. For example, at the standard stimulator responder ratio of 1:1 and an early time point (day 4), bulk spleen triggered a proliferative response of 10^5 cpm in CD4⁺ cells but only 10^4 cpm in

TABLE I
Development of Allospecific CTL in the Dendritic Cell – CD8⁺ Lymphocyte MLR

Exp.	MHC (H-2) of:		⁵¹ Cr target	Percent specific ⁵¹ Cr release at E/T ratios of:		
	Stimulator	Responder		30:1	10:1	3:1
1	H-2b × H-2d	H-2d × H-2d	H-2 b × d	28	20	1.6
			H-2 d × d	0	0	0
2	H-2b × H-2d	H-2d × H-2d	H-2 b × d	15:1	5:1	1.5:1
			H-2 d × d	29	18	11
3	H-2d × H-2d	H-2k	H-2 b × d	0.7	1	0.7
			H-2 d × d	50:1	15:1	5:1
			H-2 k	49	29	17
				0	0	0

MLR cultures, each containing 4×10^4 dendritic cells and 4×10^6 CD8⁺ lymphocytes in 1 ml culture medium, were run in triplicate. 25% vol/vol of GK 1.5 anti-CD4 culture supernatant was added to block any residual CD4⁺ function, as verified by running companion CD4⁺ MLRs (see Fig. 4). At 96 h the cells were washed and added in graded doses to 10^4 ⁵¹Cr-labeled peritoneal macrophages from stimulator or responder mouse strains (20). Specific lysis was measured at 4 h. Spontaneous ⁵¹Cr release was <20%. Lysis in the syngeneic MLR was 0–5% (not shown).

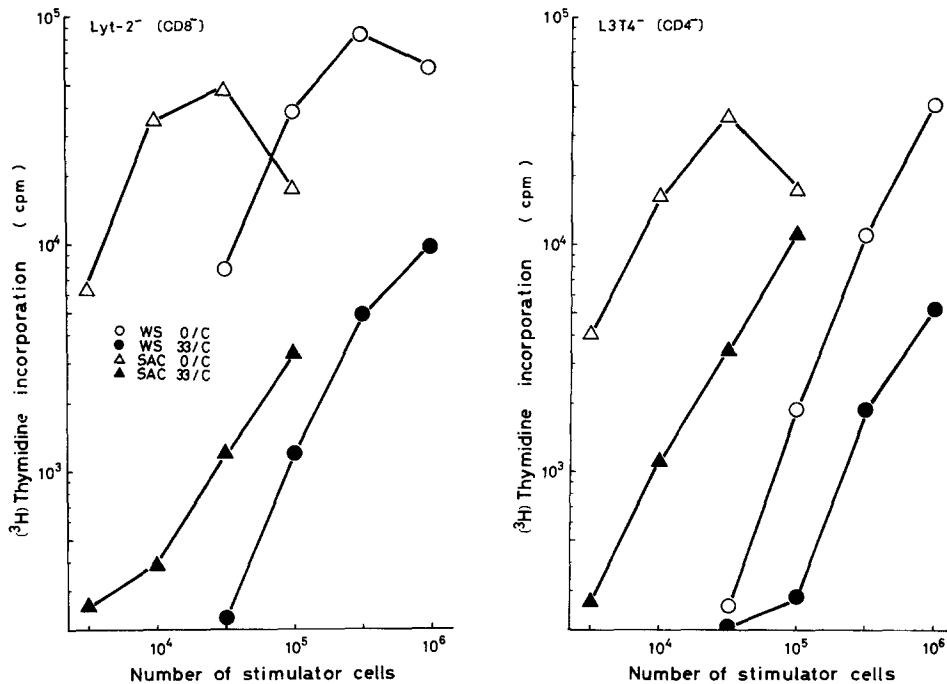


FIGURE 5. Dendritic cells are the main stimulator of CD4⁺ and CD8⁺ MLRs. Heterogeneous leukocytes (whole spleen suspensions, WS; or spleen adherent cells, SAC) from B6xD2 mice were treated with no mAb and complement or 33D1 anti-dendritic cell mAb and complement, washed, irradiated (900 rad), and added in graded doses to 3×10^5 CD4⁺ (Lyt-2⁻) or CD8⁺ (GK 1.5⁻ or L3T4⁻) lymphocytes from CxD2 mice. The MLRs were measured at 86–96 h. Since dendritic cells were a trace component of both WS and SAC populations, killing was not detectable by trypan blue exclusion. Nevertheless, the dose-response curves indicated that most of the stimulatory function was ablated in this and two other experiments.

TABLE II
Relative Efficacy of Dendritic Cells and Macrophages as MLR Stimulators

Stimulators	Proliferative response to graded doses stimulator cells					
	10^5	3×10^4	10^4	3×10^3	10^3	3×10^2
CD4⁺ responders						
Spleen adherent cells	25.4	67.5	32.0	3.7		
Dendritic cells	87.6	186.5	232.0	164.1	43.6	9.4
Peritoneal macrophages	0.9	1.4	0.4	0.3		
CD8⁺ responders						
Spleen adherent cells	17.8	39.3	17.0	2.3		
Dendritic cells	44.7	61.0	42.8	36.8	5.7	1.4
Peritoneal macrophages	0.5	0.5	0.5	0.3		
plus 10^4 dendritic cells	10.9	15.5	24.8	33.2		
plus 10^3 dendritic cells	12.6	19.1	24.3	29.3		
plus 10^2 dendritic cells	7.1	9.5	11.5	12.3		

Graded doses of B6 \times D2 F1 stimulators were added to 3×10^5 C \times D2 F1 T cell subsets (Lyt-2⁻ or CD4⁺, top, and L3T4⁻ or CD8⁺, bottom). Proliferation was measured at 112–120 h. Dendritic cells were >10 times more active as stimulators than spleen adherent cells (<10% dendritic cells). Peritoneal macrophages were not stimulatory but could inhibit the function of dendritic cells.

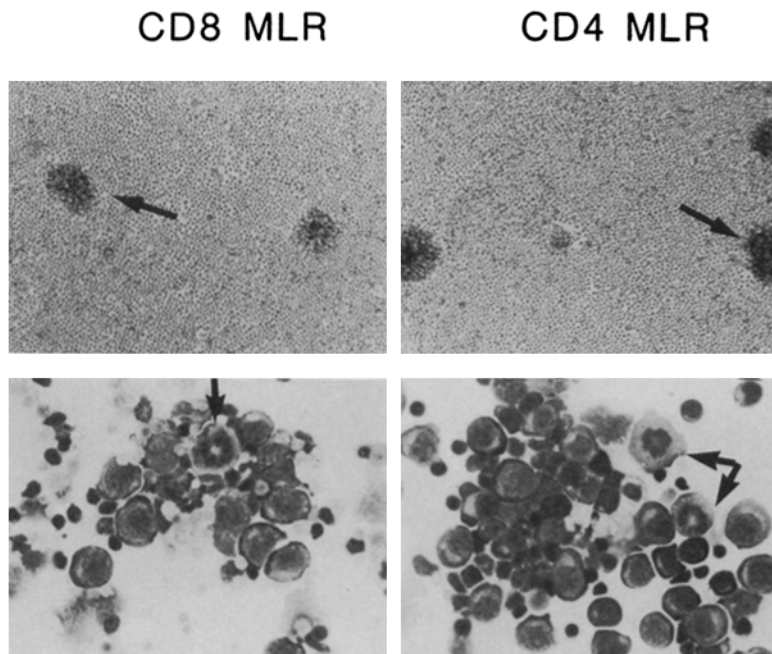


FIGURE 6. Cluster formation during DC-stimulated MLRs. On the top are low-power views of the MLRs at 24 h in an inverted microscope. When dendritic cells were added to CD4⁺ or CD8⁺ lymphocytes, clusters were readily evident (*arrows*). Clusters were not observed when dendritic cells or responding T lymphocytes were cultured separately, when CD8⁺ lymphocytes were challenged with syngeneic dendritic cells, or when allogeneic macrophages and B cells were used as stimulator cells (not shown). On the bottom are Giemsa stains of a representative cluster isolated by velocity sedimentation at 24 h, cultured one more day, and sedimented onto a glass slide. Note the large numbers of lymphoblasts, including some in mitosis (*arrows*).

TABLE III
Dendritic Cells-T Clusters Are the Principal Site of the CD4⁺ and CD8⁺ MLRs

Parameter	CD4 ⁺ T cells			CD8 ⁺ T cells		
	Cluster	Noncluster	Mix	Cluster	Noncluster	Mix
Percent DC in each fraction at 24 h	3.3	0	—	2.3	0	—
Number of cells in each fraction at 24 h ($\times 10^{-6}$)	0.25	2.7	—	0.13	1.8	—
Proliferative response (cpm $\times 10^{-3}$) at:						
36-48 h	18.9	2.9	28.0	33.9	1.6	36.1
66-72 h	102.6	0.7	176.2	150.0	0.9	180.0
90-96 h	151.0	0.4	284.4	48.8	0.9	70.9
114-120 h	42.4	0.4	95.3	2.1	0.6	12.4

5×10^4 B6.H-2k DC were labeled with a carbocyanine dye (22) and added to $C \times D2$, H-2d, CD4⁺, or CD8⁺ T cells (5×10^6 per culture) for 24 h. Cluster and noncluster fractions were isolated by velocity sedimentation. Cell numbers and percent dendritic cells were counted on a hemocytometer. Aliquots of 3×10^4 clustered cells, 1.5×10^5 nonclusters, or mixtures were replated in microwell cultures, and proliferation was measured at the times indicated.

CD8⁺ cells (Fig. 5). The use of bulk stimulator populations would therefore make it difficult to detect a CD8⁺ MLR.

Enriched populations of dendritic cells were then compared with enriched peritoneal macrophages as stimulators of the CD8⁺ MLR. Resident and thioglycollate-elicited macrophages did not initiate an MLR (Table II). Higher doses (10^3 - 10^4) of macrophages could inhibit stimulation by dendritic cells (Table II). We conclude that to generate responses from CD8⁺ populations, transplantation antigens are best administered by dendritic cells rather than other leukocytes.

Clustering of Dendritic Cells and Responding CD8⁺ T Lymphocytes. An important feature of immune responses is that the accessory dendritic cells cluster the responding T lymphocytes for prolonged periods (one or more days). The subsequent immune response develops from these cell clusters (21, reviewed in reference 5). Other APCs, such as macrophages and B lymphocytes, bind to sensitized but not resting CD4⁺ cells (21, 28). When CD4⁺ and CD8⁺ MLRs were induced by dendritic cells, comparable clustering was evident (Fig. 6, *top*). If clusters were isolated at 20 h, and cultured another day, most of the clustered lymphocytes were typical activated lymphoblasts (Fig. 6, *bottom*). Clustering was not evident when CD4⁺ or CD8⁺ lymphocytes were stimulated with allogeneic lymphocytes or macrophages (not shown). Quantitative studies indicated that most of the dendritic cells and 5-10% of the lymphocytes were clustered in 24 h (Table III). When cluster and noncluster fractions were returned to culture, proliferation (Table III) and cytolytic T cell formation (not shown) were enriched in the clusters, which functioned at very low cell concentrations. In different experiments, proliferation by clustered lymphocytes approached (Table III) or equalled (not shown) the mixture of clusters and nonclusters. Therefore clusters are the principal site for the CD8⁺ MLR, and these clusters seem to be uniquely induced by dendritic cells.

Discussion

The observations in this paper reveal that a remarkably similar pathway leads to the activation of isolated CD8⁺ and CD4⁺ T lymphocytes during responses to foreign leukocytes. Transplantation antigens are "sensed" by T lymphocytes when presented on lymphoid dendritic cells that efficiently bind and activate CD8⁺ cells to become lymphoblasts with modest cytolytic activity. Dendritic-T cell (CD4⁺ or CD8⁺) clusters do not form when antigens are presented on foreign macrophages and lymphocytes, so that it is possible that the presence of antigen is being ignored by the T cells. The clustering of dendritic and T cells may require an antigen-independent binding mechanism that recently has been described (28). Clustering could have several consequences. It could provide the time required for products on two opposing cell surfaces, particularly MHC molecules on the presenting cell and receptors on unprimed lymphocyte clones, to align and interact. Clustering could also provide "second signals," that is stimuli other than antigen, that are necessary for T cell activation.

In contrast to resting T cells, the sensitized T blast can bind and respond to antigens on other presenting cells. This is illustrated by the data on allogeneic peritoneal macrophages. Peritoneal macrophages express readily detectable levels of the class I MHC products that are recognized by CD8⁺ cells. Expression of macrophage class I antigens has been monitored using cytotoxicity and alloantibodies (29) as well as binding and immunoprecipitation with monoclonal reagents (19, 30). Macrophages do not stimulate the CD8⁺ subset, yet serve as excellent targets for the CTL that are induced by dendritic cells (Tables I and II). Likewise, Con A-induced lymphoblasts do not stimulate an MLR (Metlay, J., and R. Steinman, unpublished observations) but are standard target cells for sensitized CTL. Therefore, immune recognition, as evidenced by APC-T cell binding or T cell responsiveness, clearly differs with the state of the T lymphocyte. Unprimed and memory lymphocytes bind and respond to dendritic cells, while primed T blasts can interact with many types of presenting cells (28).

Of some interest is the fact that production of IL-2 is readily detected in the dendritic-CD8⁺ MLR (Fig. 2). There has been a wide range of reported results on the ability of CD8⁺ cells to make T cell growth factors, presumably IL-2. Some studies fail to detect IL-2, others add anti-IL-2-R antibodies to reduce IL-2 utilization (13), while others (31-33) readily detect the lymphokine. The results of von Boehmer et al. (32) may be particularly relevant, since CD8⁺ T cells frequently lose the capacity to release growth factor after prolonged growth in culture. Another possibility is that a subset of CD8⁺ cells produces abundant IL-2, and this subset requires dendritic cells to be activated. Examination of individual CD8⁺ cells in dendritic-CD8⁺ clusters (Fig. 6), as by *in situ* hybridization, may clarify the frequency of CD8⁺ cells that produce IL-2 initially. Recent data (23) indicate that mitogens induce comparable levels of IL-2 mRNA in primary CD4⁺ and CD8⁺ responses.

Where CD4⁺ and CD8⁺ MLRs differ considerably is in the longevity of the proliferative response (Fig. 1), as reported by Sprent and Schaefer (12). The fact that newly formed CD8⁺ blasts can kill APCs may explain the different kinetics. Lysis of APCs may also account for the observation that large numbers of allogeneic stimulators must be applied when whole spleen is the source of antigen

(Fig. 5). In the CD4⁺ MLR, the lymphoblasts that are sensitized in the first 3–4 d of culture can continue to respond to the excess of allogeneic macrophages and B cells that remain in the culture (21). In the CD8⁺ MLR, lymphoblasts are induced, but additional rounds of proliferation will not occur if the presenting cell is killed by the sensitized CTL. We had noted this previously when we compared the restimulation requirements of Lyt-2⁻ and Lyt-1⁻ T blasts (reference 21; Table IV). The former responded vigorously to B cells, but the Lyt-1⁻ blasts killed the lymphocytes and did not respond at all.

While helper cells are not required for the initiation of CTL responses to antigens on dendritic cells, helper cells may be critical for sustaining and amplifying CTL responses after the presenting cells are killed. If we isolate CD8⁺ T blasts from the MLR at days 3 or 4, exogenous factors must be added to retain and expand CTL activity in the ensuing 1–2 d of culture (our unpublished observation). We find that these factors are abundant in the conditioned medium of the CD4⁺ MLR.

Of some interest is the role of dendritic cells in the primary response to viral and tumor antigens on other cells. Are antigens on infected or malignant cells presented directly to unprimed CD8⁺ T lymphocytes or via a dendritic cell in the host? A pathway of CD8⁺ CTL activation, in which viral antigen is presented on dendritic cells, represents a potential but unexplored resistance mechanism for acquired immune deficiency syndrome in which functioning CD4⁺ helper cells are compromised, or for any agent in which determinants can be presented in association with class I but not class II MHC products. Again it needs to be shown that dendritic cells can present such antigens. For these reasons, the results described in this report are being extended to human leukocyte cultures and to the generation of virus-specific CTL.

Summary

Recent experiments (11–13) have shown that antigen-specific, CD8⁺,CD4⁻ T lymphocytes can be induced to proliferate and become killer cells in the absence of a second population of “helper” CD8⁻,CD4⁺ cells. We have studied early events in the activation of CD4⁺ and CD8⁺ T cell subsets in the primary mixed leukocyte reaction. Dendritic cells are a major if not essential accessory cell for the activation of both subpopulations. Antigen-bearing macrophages fail to stimulate unprimed CD8⁺ cells, but act as targets for the sensitized cytolytic lymphocytes that are induced by dendritic cells. The initial proliferative response is comparable for CD4⁺ and CD8⁺ lymphocyte subsets. For both subpopulations, dendritic cells efficiently cluster the responding lymphocytes on the first day and induce the release of IL-2. The data indicate that CD4⁺ and CD8⁺ lymphocytes can be activated by a similar mechanism, and illustrate the special role of dendritic cells in the sensitization stage of cell-mediated immunity.

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