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AN ANTIGEN-INDEPENDENT CONTACT MECHANISM AS AN EARLY STEP IN T CELL-PROLIFERATIVE RESPONSES TO DENDRITIC CELLS

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The administration of an antigen to the immune system can lead to the selection and expansion of clones precommitted to respond to that antigen. Antigens are not presented directly to T lymphocytes. Instead, antigens are processed and complexed to transmembrane products of genes in the MHC. The antigen-MHC complexes then are presented on the surface of APC (1–4).

How might antigen-MHC complexes on the surface of APC find and select clones of T cells that are specific to that antigen, since neither the ligand nor the TCR are free to diffuse in solution? Also, the amount of peptide/MHC complex and the frequency of antigen-specific T cell clones both may be small. Nonetheless, it is evident that antigens on dendritic cells are capable of selecting specific T lymphocytes from a pool of lymphocytes in culture. For example, on the first day of a primary MLR, most of the antigen-specific T cells have formed clusters with dendritic cells (5, 6). The clustered lymphocytes then proliferate and release lymphokines. The specificity of the dendritic-T cell binding is evident by the facts that (*a*) the nonclustered population is selectively depleted of antigen-reactive T cells; and (*b*) the lymphoblasts that are derived from the clusters bind and respond to those dendritic cells bearing the original stimulating antigens (5–7). Likewise, in primary T-dependent antibody responses to red cells and hapten-carrier conjugates, most of the antibody-secreting cells arise from clusters of interacting dendritic cells, B, and T lymphocytes (8, 9). During the antibody response, Th cells may first be bound by the dendritic cell; then the activated helper cell binds the antigen-specific B cell (8, 10).

To bring about clonal selection, it has been hypothesized that dendritic cells exhibit an antigen-independent but reversible binding mechanism for surveying resting T lymphocytes (7, 11). T cells are retained in these cell aggregates as long as there is complementarity between the presented antigens and the TCR. Antigen-independent binding between dendritic cells and T lymphocytes has been demonstrated using T lymphoblasts as test cells (7). Here we describe a model that indicates that such an antigen-independent mechanism occurs with resting T cells.

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The model involves certain dendritic cells, those from thymus and skin (Langerhans cells [LC][1]), which have small but nonetheless significant levels of one type of receptor for immune complexes, type II Fcγ receptors (FcγRII) (12). FcR on the APC present anti-CD3 mAb to the CD3 portion of the TCR complex, thus bypassing the α/β clonotypic portion of the TCR and providing an intense polyclonal stimulus (13). Even though LC have only small numbers of FcR, ~2,000 per cell, the LC is a potent APC for anti-CD3 responses (12). One would assume that the FcR and anti-CD3 substitute for antigen on APC and function early on to "oponsize" or bind the T cell to the LC. However, we will present evidence that the initial binding of large numbers of T cells to dendritic cells does not require either FcR or the anti-CD3 mAb, suggesting that dendritic cells can "survey" T cells by an antigen-independent mechanism. We also describe two-chamber experiments indicating that dendritic-T cell contact is essential for mitogenesis.

Materials and Methods

Mice. Male or female BALB/c × DBA/2 F_1 mice were purchased from the Trudeau Institute, Saranac Lake, NY and used at 6–9 wk of age.

mAbs. 2.4G2 anti-FcγRII (14), S4B6 anti-IL-2 (15), F4/80 (16) and M1/70 (17) antimacrophage, B21-2 (18) and M5/114 (19) anti-MHC class II or anti-Ia, GK1.5 anti-CD4 (20), 53-6.7 anti-CD8 (21), F441.8 anti-CD11a/LFA-1 (22), and 11B11 anti-IL-4 (23) were used as hybridoma culture supernatants; 2C11 anti-CD3 (24) as ascites.

Dendritic Cells. Thymi were digested with collagenase. Low density cells, corresponding to 2–5% of the total, were isolated on albumin columns (25). An adherent low density fraction, containing macrophages and dendritic cells, was prepared and cultured overnight. Cells that eluted from the culture surface were depleted of residual macrophages by rosetting with antibody-coated red cells, providing a dendritic cell-enriched fraction that was >90% pure by morphology and surface markers, i.e., abundant class I and II MHC products and absence of B, T, and macrophage antigens (25). LC were enriched from fresh or cultured epidermal cell suspensions as described (12, 26, 27). In most cases, the experiments utilized 3-d cultured LC that were enriched to 50–80% purity on albumin columns (26), but for some experiments (Results; Table 2), the LC were purified to >80–90% purity using a panning technique (27). Both thymic and epidermal dendritic cells did not bind antibody-coated RBC, but they did express small amounts of FcγRII as detected by a sensitive FACScan flow cytometer or by binding of ^{125}I-anti-FcR mAb (12).

T Cells. Nylon wool–nonadherent mixtures of spleen and lymph node cells were treated with anti-Ia and complement and pelleted in discontinuous Percoll gradients (45:54:63%) to provide accessory cell–depleted populations (12). The high density Ia^- T cell populations showed little or no background proliferation to the mitogens con A or anti-CD3 mAb, even when cultured at 2–3 × 10^5 cells/microwell (see Results).

APC-T Cell Clustering and T Cell Mitogenesis. Dendritic cells were mixed with T cells at a ratio of 1:50 in the MLR (H-2^d dendritic cells stimulating H-2^k T cells) (5) or 1:80–1:100 in the presence of 2C11 hamster anti-mouse CD3 (12). 5–10 × 10^6 T cells were cultured in polystyrene tubes (2057; Falcon Labware, Oxnard, CA) in 1 ml of RPMI 1640 supplemented with 5% FCS (Hazelton Systems, Inc., Aberdeen, MD) 20 μg/ml gentamicin, and 50 μM 2-ME. For peritoneal exudate macrophages, tubes with minimal protein absorption (Minisorp, 466982; Nunc, Roskilde, Denmark) were used. 3–12 h after mixing APC and T cells, the contents were gently resuspended by swirling and two tubes were applied per column of 5 ml 50% FCS-RPMI 1640. After sedimentation at 1 *g* for 1 h at 4°C, cell clusters pelleted to the bottom 1 ml of the column. Cluster and noncluster fractions corresponding to 10–20% and 30–40% of the applied cells were obtained. To minimize handling of the clusters, which could be dissociated by pipetting, the bottom 1 ml of the column with the clusters was diluted

[1] *Abbreviation used in this paper:* LC, Langerhans cells.

to 10 ml or more with culture medium and distributed in graded doses. The culture vessels were either microtest wells (25860; flat-bottomed, Corning Glass Works, Corning, NY; 76-013-05 round-bottomed, Flow Laboratories, McLean, VA) with 0.2 ml total medium, or macrotest wells (25820; Corning Glass Works) containing 0.4-μ millipore filter inserts (PIHA 01250; Millipore Continental Water Systems, Bedford, MA). 0.5 ml of the cell suspension was placed in the top chamber and 0.7 ml in the bottom, at doses indicated in Results. DNA synthesis was monitored by adding 1–4 μCi of [^{3}H]TdR (6 Ci/mmol; 4 μCi/ml final concentration) for 8–16 h.

T Cell Growth Factors. Production of growth factors from APC-T cell clusters was measured using either the CTLL-2 cell line or con A-induced lymphoblasts as the responding T cell. To determine the relative contribution of IL-2 vs. IL-4 as growth factors, we added the S4B6 (15) or 11B11 (23) neutralizing anti-IL-2 and anti-IL-4 mAb, respectively.

Cytologic Assays. After separation on serum columns, the clusters were spun onto glass slides (Cytospin 2; Shandon Southern Instruments Inc., Sewickley, PA). After fixation in acetone for 5 min at room temperature, the slides were exposed successively to hybridoma culture supernatants (see above), biotin-rabbit anti-rat Ig, and avidin-biotin-peroxidase complex (Vector Laboratories, Inc., Burlingame, CA). The peroxidase was visualized with diaminobenzidine-hydrogen peroxide. Nuclei were counterstained with hematoxylin.

Results

Anti-CD3-induced T Cell Proliferation Occurs in APC-T Cell Clusters. The 2C11 anti-CD3 mAb (24) and small numbers of APC were added to cultures of T lymphocytes from spleen and lymph node, at a ratio of 1:100. Dendritic cells from thymus or epidermis were compared with peritoneal macrophages. Within 2–4 h, most of the APC had formed aggregates with ~20% of the T cells. Clustering was assessed by separating aggregates and nonaggregates by 1-*g* sedimentation and then immunolabeling with mAb to the surface of the APC (Fig. 1). The clusters that formed with dendritic cells or macrophages appeared similar at the light microscope level. Most of the aggregates contained more than one APC, and the ratio of lymphocytes to APC was ~10–20:1. Both CD4 and CD8 T cells were represented in the clusters (not shown).

When the cell clusters were returned to culture, active DNA synthesis was observed within 1 d and was dependent upon the continued presence of the 2C11 anti-CD3 mAb (Fig. 2, compare closed and open symbols). Large responses were generated by small numbers of clustered cells in 6-mm microtest wells, e.g., 5,000 cells as compared with standard doses of 300,000 T cells that are used to study mitogenesis (Fig. 2). Many of the clustered lymphocytes became enlarged lymphoblasts, and many mitotic figures were observed (not shown).

In the above experiments, the T cells that had not formed clusters at 3–4 h were recultured with or without a fresh inoculum of dendritic cells or macrophages. Regardless of the APC that was used to form the clusters initially, isolated nonclustered cells did not proliferate. However, the nonclustered cells were as responsive as bulk T cells to a fresh inoculum of either dendritic cells or macrophages (Fig. 3).

Antigen-independent Dendritic-T Cell Cluster Formation. Previously it was shown that the mitogenesis response to anti-CD3 mAb was blocked with the 2.4G2 anti-FcRγII antibody, and that this reagent acted at the level of the APC rather than the T cell (12, 28). One might expect that FcγRII would be required for the initial binding of the T cell to the APC, as occurs in classical opsonization of bacteria and cells to phagocytes.

However, 2.4G2 did not block binding of T cells to dendritic cells in the presence

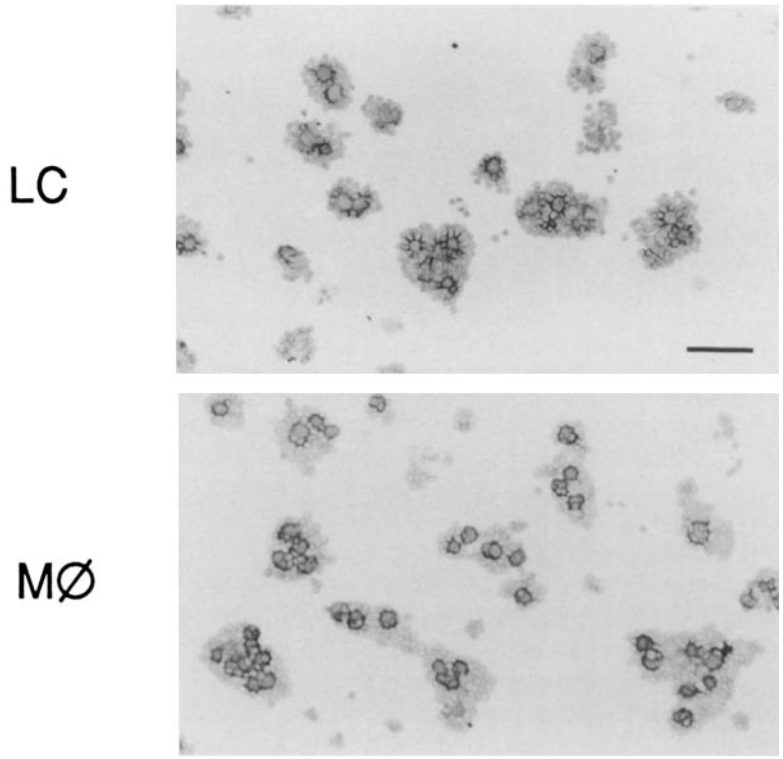

FIGURE 1. Clustering of APC with T lymphocytes. Cultured LC (*top*) or peritoneal cells containing 30% macrophages (*bottom*) were cultured with T cells at an APC/T ratio of 1:100 or 1:30, respectively, for 3 h in the presence of anti-CD3. Clusters were separated by 1-*g* sedimentation, cytospun onto slides, immunolabeled with mAb to identify the APC, and counterstained with hematoxylin to show the T cells. The mAbs were B21-2 anti-I-A for dendritic cells, or a combination of F4/80 and M1/70 for macrophages. Bar, 0.09 mm (× 81).

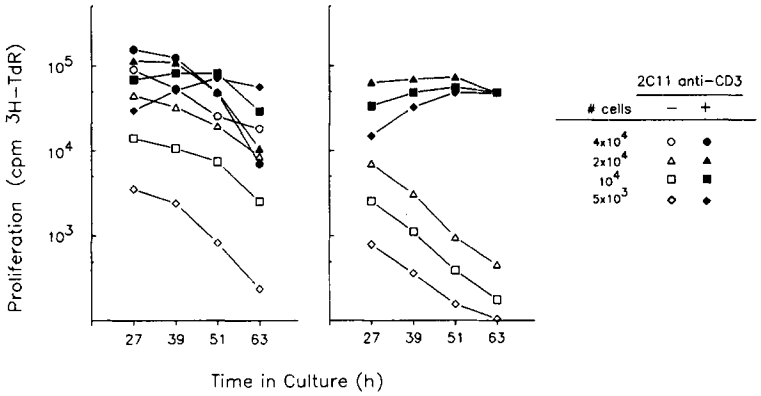

FIGURE 2. Proliferative responses to anti-CD3 occur in APC-T cell clusters. Thymic dendritic cells (*left*) or peritoneal macrophages (*right*) were added together with 2C11 anti-CD3 ascites (plateau dose of 1:2,000) to T lymphocytes for 4 h. Clusters were isolated and cultured at the indicated doses in microtest wells with (*closed symbols*) or without (*open symbols*) further anti-CD3 mAb. 1 μCi [^{3}H]TdR was added for 6 h, and replicate cultures were harvested to assay [^{3}H]TdR uptake at the indicated times.

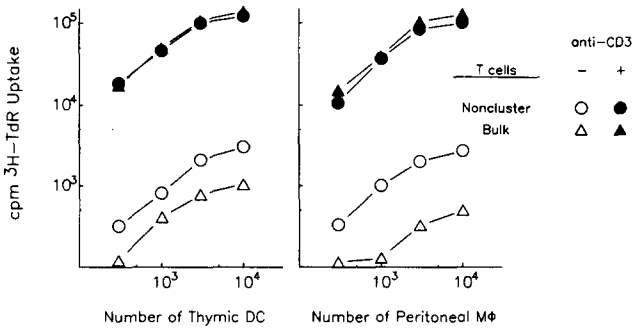

FIGURE 3. Nonclustered T lymphocytes are fully responsive to APC. Thymic dendritic cells (*left*), peritoneal macrophages (*right*), or LC (not shown) were cultured with T lymphocytes and anti-CD3 mAb for 3 h. Cluster and noncluster fractions were isolated. The nonclusters, as well as a sample of bulk T cells that had not been previously exposed to APC, were cultured at 2×10^5 cells/microtest well in the presence of the indicated doses of fresh accessory cells. In the absence of APC, the nonclustered and bulk T cells showed only 400–800 cpm of [^{3}H]TdR uptake, which was measured at 28–32 h. The APC-T cell clusters, at 10^5 cells/well, exhibited 1.2–1.5 $\times 10^5$ cpm uptake (not shown). Also not shown are "heterologous" APC data, in which macrophages were used to restimulate the nonclusters from thymic DC and vice versa; these T cells gave the same response as the "homologous" systems shown here.

of anti-CD3 mAb; in fact, comparable clustering occurred in the presence or absence of anti-CD3 (Fig. 4, *A–C*). Clustering did not occur when dendritic and T cells were mixed on ice however (not shown). In contrast to dendritic cells, macrophage-T cell binding was blocked by anti-FcγRII and required anti-CD3 (Fig. 4, *D–F*).

To prove that comparable numbers of responsive lymphocytes had bound to dendritic cells in the presence or absence of anti-CD3 or anti-FcγRII, the clusters that had formed under each of the above conditions (−/+ anti-FcR, −/+ anti-CD3) were isolated on velocity gradients in two experiments. About 30% of the total cells in the culture were found in the cluster fractions formed in the presence or absence of anti-FcR and/or anti-CD3. Each set of clusters proliferated comparably when returned to culture, and this proliferation was dependent upon FcR and anti-CD3 (Table I). We conclude that a ligand for the TCR (FcR and anti-CD3), while not essential for clustering, is required for DNA synthesis. We therefore refer to the initial binding mechanism that brings dendritic cells and large numbers of T lymphocytes together as antigen independent. The T cells most likely are resting lymphocytes since they are small, high density, and IL-2 unresponsive (see below).

The T Cell Binding Mechanism of LC Matures in Culture. Prior observations indicated that epidermal dendritic cells (LC) in freshly prepared suspensions were weak accessory cells for T cell-proliferative responses to transplantation antigens and polyclonal mitogens (26, 29). Strong accessory function developed when the LC were cultured for 2–3 d in the presence of keratinocytes or their product, granulocyte/macrophage-stimulating factor. We tested if the capacity to bind resting T cells also developed in culture, using the anti-CD3 response as a way to stimulate the T cell independent of antigen-presentation.

When fresh LC were mixed with T cells in the presence of anti-CD3, clusters were formed (Fig. 5). These clusters could be isolated on velocity gradients, but they were unstable and would disassemble upon handling, or shortly after being placed into culture. The fact that the clusters with fresh LC were so weak was striking, since fresh LC have 10 times more FcRII than cultured LC (12). When the experi-

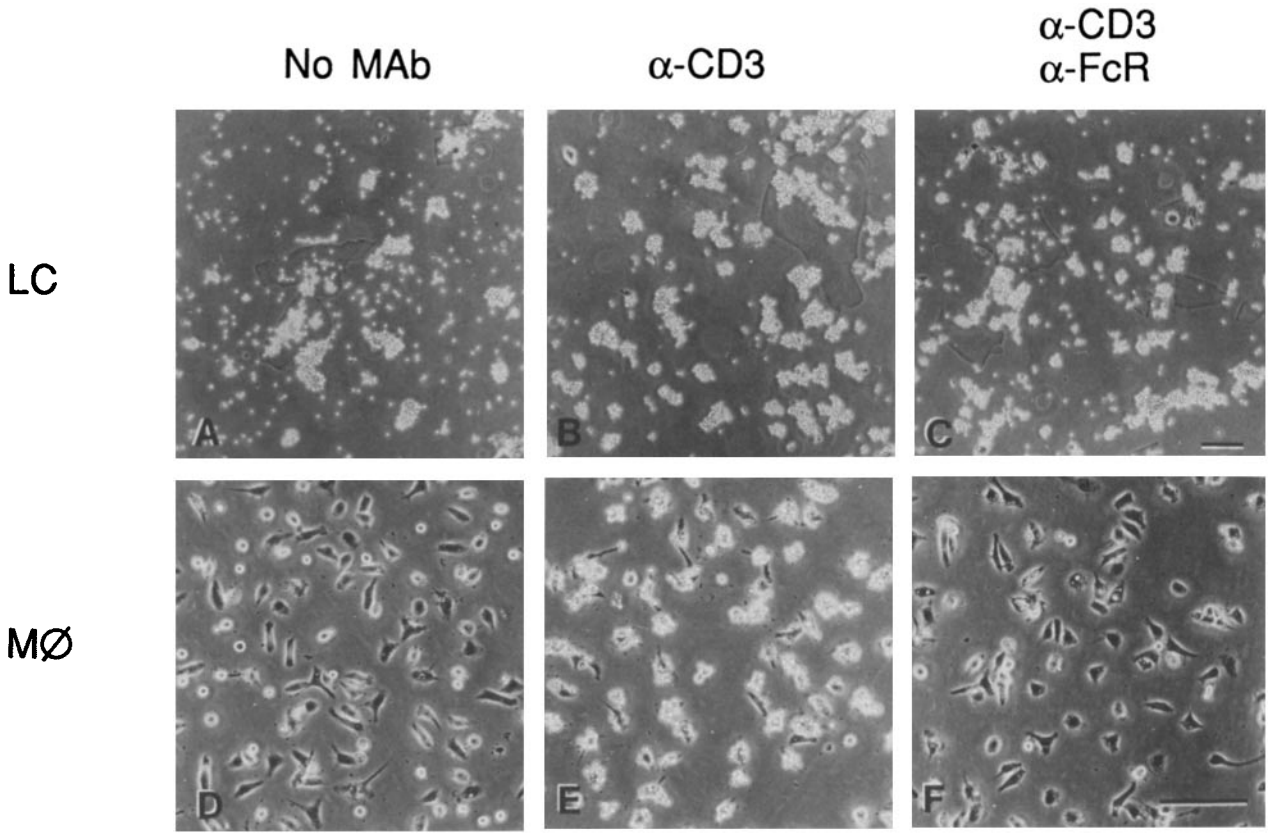

FIGURE 4. Antien-ir ependent binding of dendritic cells to T lymphocytes. (*A–C*) LC were cultu l with T lymphocytes at a dose of 1:80 for 3 h −/+ anti-CD3 (mAb 2C11 and or anti-FcR II (mAb 2.4G2). The tubes were swirled gently, fixed in 5% glutaraldehyde, and transferred to culture dishes for photography. No cl ters formed without APC (not shown). Bar, 0.07 mm (× 50). (*D–F*) Adherent peritoneal macrophages were used as APC. Here, binding of T cells requires anti-CD3 and FcR II. Bar, 0.07 mm (× 100). *Left panels*, No mAbs added; *middle panel*, anti-CD3; *right panel*, anti-CD3 and 2.4G2.

TABLE I

Anti-CD3 and FcR Are Required for Mitogenesis, not Clustering, of Dendritic Cells and T Lymphocytes

mAb during clustering (0–3 h)	mAb during mitogenesis (3–38 h)		DNA synthesis with clustered cells at:			
	α-FcR	α-CD3	8×10^4	4×10^4	2×10^4	10^4
			cpm [^{3}H]TdR × 10^{-3}			
None	–	–	4.1	2.2	9.7	7.6
	–	+	177.1	124.4	49.6	19.1
	+	–	17.9	19.6	14.0	1.9
	+	+	80.0	15.4	2.6	0.4
α-CD3	–	–	170.1	112.0	59.3	26.8
	–	+	178.3	117.5	67.9	31.1
	+	–	48.7	25.6	11.6	4.1
	+	+	37.2	14.0	8.9	3.7
α-FcR	–	–	4.7	2.7	0.9	0.6
	–	+	190.8	113.1	58.0	27.6
	+	–	4.8	2.3	0.8	0.4
	+	+	77.1	37.9	10.8	22.1
α-CD3	–	–	152.1	119.5	67.0	33.4
α-FcR	–	+	162.5	115.8	60.3	29.6
	+	–	79.1	49.2	21.0	12.5
	+	+	46.1	17.5	14.6	5.4

DC–T cell clusters were allowed to form ± α-CD3 and/or ± α-FcRII mAb for 3 h. Clusters were isolated on 1-*g* velocity columns. The yields of clusters were identical. The antibodies were removed by two washes using low speed centrifugation (80 *g*, 4 min). The clusters were resuspended gently in culture medium and cultured at the indicated doses in round-bottomed wells ± αCD3 mAb and/or ± α-FcRII [^{3}H]TdR was added at 17–29 h to measure DNA synthesis. Note that all clusters proliferated similarly in the presence of α-CD3 irregardless of the presence or absence of α-CD3 and α-FcRII during clustering (left column).

ments were repeated with LC that had been cultured overnight, the clusters that formed with anti-CD3 mAb were still less stable than those formed with 3-d cultured LC.

The function of LC-T cell clusters was assessed by monitoring DNA synthesis of comparable numbers of clustered cells that had been formed with anti-CD3 plus LC that had been cultured for 0, 1, or 3 d. The clusters formed with 3-d LC proliferated the most actively (Table II). In each case, the observed synthesis of DNA was blocked by 2.4G2 mAb. These results indicate that 3-d cultured LC have developed the ability to retain and stimulate T cells in the anti-CD3 system. This functional property of the LC increases as the expression of needed FcγRII decreases.

Two-chamber Experiments to Show that APC-T Cell Contact, Rather than Lymphocyte-activating Factors, Are Required to Initiate Mitogenesis. To determine if dendritic-T cell clustering was essential for mitogenesis, or was simply required to release required lymphocyte-activating factors, we performed two-chamber experiments. Clusters of dendritic

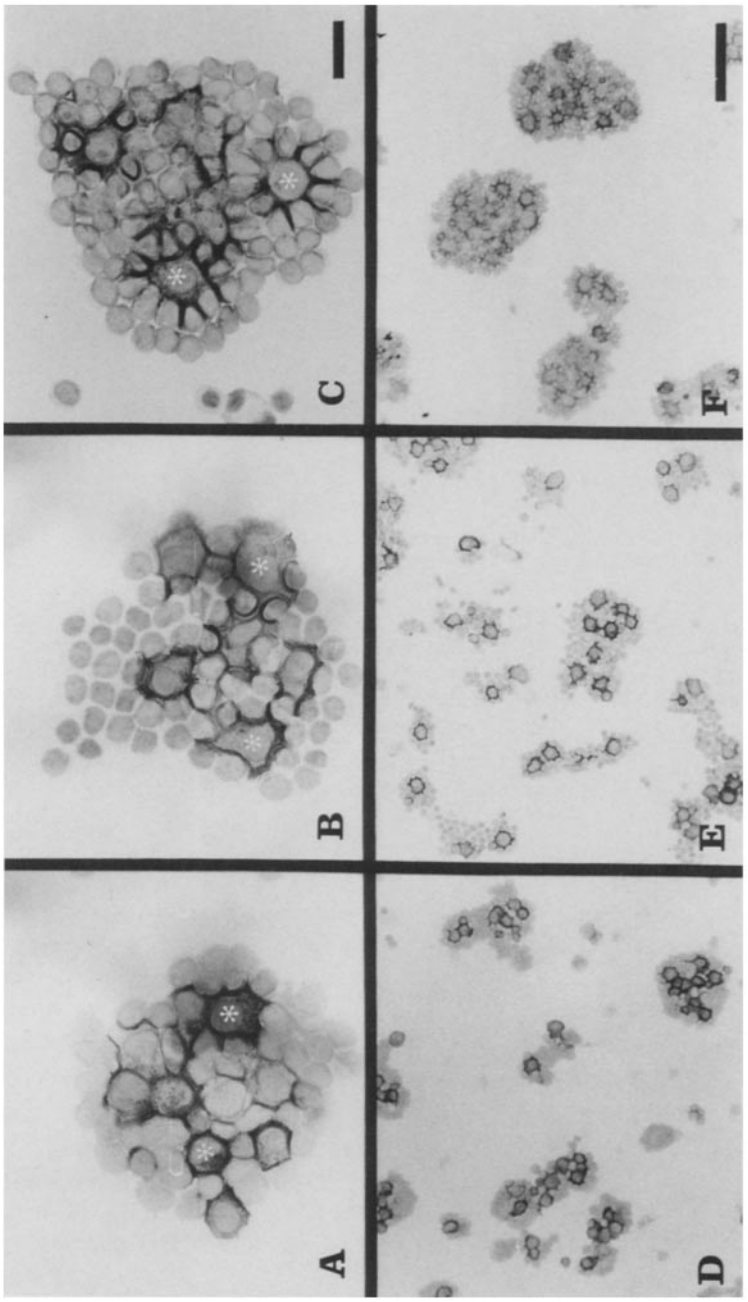

FIGURE 5. Clustering by LC isolated at different periods of epidermal culture. LC were enriched after 0 (*A*, *D*), 1 (*B*, *E*), or 3 (*C*, *F*) d of coculture with keratinocytes, during which time it is known that the accessory function of these dendritic cells for T cell proliferation increases markedly (26, 27) (Table IV). LC were selected by a panning method (27) after 0, 1, or 3 d of culture, and then were allowed to cluster T cells in the presence of anti-CD3. The clusters were isolated for immunolabeling with anti-Ia mAb as in Fig. 1. One of the Ia^+ LC in each cluster is marked by an asterisk. *A*, *B*, and *C* (× 300); and *D*, *E*, and *F* (× 100).

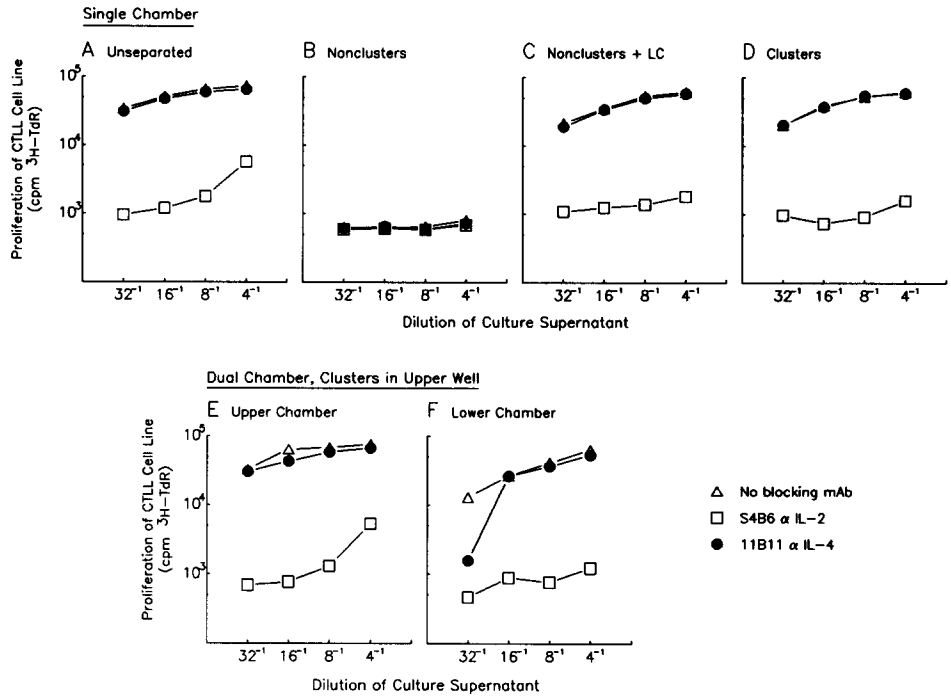

FIGURE 6. T cell growth factor release from dendritic-T cell clusters. Thymic dendritic cells were cultured for 3 h with syngeneic T cells at a ratio of 1:50 in the presence of 2C11 mAb in polyethylene tubes. Several cell populations (see headings, *A–D*) were then cultured for 11 h and the medium was harvested for assay of T cell growth factor activity either on the CTLL cell line (shown here) or con A-induced lymphoblasts (not shown). In addition, clusters were isolated and 3 × 10^5 cells added to the upper chamber of dual-chamber wells. The medium in the upper (*E*) and lower (*F*) chambers was taken at 11 h and tested in the bioassay. To identify the growth factor, we tested if the activity was inhibited by S4B6 anti-IL-2 and 11B11 anti-IL-4.

cells and T lymphocytes were placed in one chamber and T cells in the other. We first checked that the APC-T cell aggregates released growth factors that could cross the 0.4-μ filter into the second chamber. Growth factor was detected in both chambers using as a bioassay either the CTLL line (Fig. 6) or con A-induced lymphoblasts (not shown). The factor was primarily IL-2, since, in both bioassays, it was almost entirely blocked by the neutralizing anti-IL-2 mAb, S4B6, but not by anti-IL-4 mAb, 11B11 (Fig. 6).

If T cells were separated from dendritic-T cell clusters by a membrane with 0.4 μ pores, and anti-CD3 mAb was present in both chambers, the isolated T cells did not proliferate but the clustered lymphocytes did (Table III, groups *6–8*). When the T cells were added to the chamber containing the clusters, an enhanced T cell proliferative response was noted (Table III, *9–11*). This indicated that the clusters were not consuming all available growth factors. Similar results were obtained when macrophage-T cell clusters were placed in the inner chamber.

The experiments were repeated, but now nonclustered T cells were studied in addition to bulk T lymphocytes (Table IV). Nonclusters could differ from bulk T

TABLE II

Cultured LC More Effectively Stimulate Bound T Lymphocytes

LC used to form clusters	Anti-CD3 (3-31 h)	Other mAb (3-31 h)	DNA synthesis (24-31 h) by LC-T cell clusters cultured at a dose of: 10^5	5×10^4	2.5×10^4	1.25×10^4
			cpm [^{3}H]TdR $\times 10^{-3}$			
day 3 LC	–	None	144.5	105.8	79.8	36.8
	+	None	174.2	127.7	98.4	48.6
	–	α-FcR	64.0	37.5	16.6	10.4
	+	α-FcR	68.1	28.5	17.6	7.7
day 1 LC	–	None	35.5	21.4	10.8	4.9
	+	None	101.9	54.1	37.0	16.4
	–	α-FcR	3.3	1.7	0.9	0.6
	+	α-FcR	2.7	1.8	1.3	0.6
day 0 LC	–	None	4.0	1.8	1.1	0.5
	+	None	42.8	2.0	7.3	4.7
	–	α-FcR	0.5	0.5	0.3	0.3
	+	α-FcR	0.5	0.5	0.6	0.3
T only	–	None	0.3	0.4	0.6	0.3
	+	None	0.9	0.4	0.4	0.4

LC were enriched by a panning technique (27) after 0, 1, and 3 d of epidermal cell culture. The LC were mixed with purified T cells at a dose of 1:50 for 3 h at 37°C in the presence of 2C11 anti-CD3 mAb to allow clusters to form. The cells were applied to a serum gradient, to allow the clusters to sediment, and the nonclusters were aspirated. The clusters were washed twice, gently resuspended, and cultured at the indicated doses another 28 h – / + anti-CD3 – / + anti-FcRII. DNA synthesis was measured at 24-31 h.

TABLE III

T Cells Are not Activated when Separated from DC-T Clusters by a Filter

Group	Cells in: Outer chamber	Inner chamber	Proliferative in: Outer chamber	Inner chamber
			cpm [^{3}H]TdR	
1	T only	–	0.3	–
2	T + IL-2	–	0.3	–
3	Medium	T + DC, 3×10^4	0.1	97.8
4	Medium	T + DC, 10^4	0.1	68.8
5	Medium	T + DC, 3×10^3	0.1	31.3
6	T	T + DC, 3×10^4	0.4	94.9
7	T	T + DC, 10^4	0.3	66.1
8	T	T + DC, 3×10^3	0.2	33.1
9	T + (T + DC), 3×10^4	Medium	169.2	–
10	T + (T + DC), 10^4	Medium	140.2	–
11	T + (T + DC), 3×10^3	Medium	61.9	–

Thymic dendritic cells were added to the inner chamber with 3×10^5 spleen-lymph node T cells. The outer chamber contained medium (groups 3-5), 10^6 T cells (groups 6-8), or mixtures of 10^6 T cells and the contents of the inner chamber (groups 9-11). 5 μCi [^{3}H]TdR was added at 40 h and aliquots were tested for thymidine uptake at 48 h.

TABLE IV

Attempts to Demonstrate Lymphocyte-activating Factors from Dendritic-T Cell Clusters

Length of separation of dendritic-T cell clusters	Dose of DC/T clusters*	A	B	C	D	E	F	G
		Clusters ± T cells in inner chamber			Clusters separated from T cells across a millipore			
		Clusters only	Clusters + bulk T‡	Clusters + nonclusters§	Clusters only	Bulk T‡	Clusters only	Non-clusters§
4 h	2×10^5	87.1	149.6	148.2	86.1	0.3	85.6	0.5
	10^5	41.3	109.1	122.0	40.0	0.3	42.4	0.4
	5×10^4	17.4	70.6	73.9	15.9	0.2	16.9	0.3
	2.5×10^4	7.4	23.9	26.6	7.0	0.2	7.2	0.2
	1.25×10^4	3.1	8.9	10.0	2.6	0.2	2.7	0.3
	None	-	0.2	0.4	0.0	0.2	0.0	0.3
	None + 10 μ/ml rIL-2	-	0.4	0.6				
12 h	2×10^5	117.8	162.8	166.9	103.8	9.8	106.2	50.3
	10^5	74.3	122.6	137.7	63.8	0.5	60.8	57.6
	5×10^4	28.3	87.4	109.8	24.9	1.0	26.2	50.5
	1.25×10^4	3.1	10.4	16.1	2.5	0.2	2.8	10.9
	None	-	0.3	1.0	0.0	0.2	0.0	0.9
	None + 10 μ/ml rIL-2	-	0.4	63.1				

* The dose of dendritic-T cell clusters (>90% T cells) placed in the inner chamber and monitored for proliferative activity in columns A, D, and F.

‡ 10^6 bulk T cells were placed in the inner chamber with DC/T clusters in column B, or in the outer chamber across a 0.4-μ millipore from the clusters in column E.

§ 10^6 nonclustered cells were placed in the inner chamber with clusters in column C, or across a millipore in column G. The cluster and noncluster fractions were isolated at 4 and 12 h in the two parts of this experiment, which was repeated twice.

cells by having some partially activated lymphocytes that had entered and then emerged from the dendritic-T cell cluster. Cluster and noncluster fractions were isolated at 4 and 12 h of the anti-CD3 response (Table IV, *top* and *bottom*, respectively) and cultured together or across a 0.4 μ millipore.

The 4-h nonclustered fractions would proliferate when mixed directly with the clusters (Table IV *top*, columns *A–C*) but would not proliferate when separated from the clusters by a filter (Table IV *top*, columns *E* and *G*). In contrast, some of the cells in the 12-h nonclustered fractions proliferated when separated from the clusters by the filter. This level of DNA synthesis in the nonclustered fraction could be reproduced with rIL-2 (Table IV, column *G* and *bottom line*). Since T cells did not synthesize DNA in response to cluster-derived cytokines (Table III; Table IV *top*), the findings with 12-h nonclustered cells suggest that some of the lymphocytes already entered into and emigrated from the dendritic-T cell cluster between the 4- and 12-h time points. During that time, the T cell acquired responsiveness to IL-2 and did not need to encounter dendritic cells or other activating factors to continue through cell cycle.

Since the above experiments had utilized a polyclonal stimulus for the TCR, we also evaluated if antigen-specific dendritic-T cell clusters could activate other T cells across a millipore. To do this, we isolated dendritic-T cell clusters from the primary

MLR. These clusters are the site in which the response to transplantation antigens, or MHC products, develops (5, 6). The clusters were added to the upper chamber and then cultured across a millipore membrane from bulk T cells in the lower chamber. In some cases, we added allogeneic B lymphocytes as a source of transplantation antigen to the lower chamber that contained the T lymphocytes. Again, the dendritic-T cell clusters proliferated actively, but there was little or no DNA synthesis by either the B or T lymphocytes in the second chamber (data not shown). Therefore, dendritic-T cell clusters do not release factors that directly induce the growth of B and T lymphocytes.

Discussion

Usefulness of the Dendritic Cell/Anti-CD3 Model of T Cell Mitogenesis. Prior studies had shown that small numbers of FcR on epidermal dendritic cells (LC) mediate T cell proliferation in the presence of anti-CD3 mAb. It was estimated that 250 FcγRII were sufficient to drive a T cell into DNA synthesis in 24 h (12). We have pursued this system to obtain evidence that dendritic cells first loosely bind large numbers of T cells in the apparent absence of anti-CD3. The T cells that we studied were small, of high density, and unresponsive to low doses of IL-2 (Tables III and IV). The beauty of the model, we feel, is that one is stimulating large numbers of resting T cells with a known ligand of the TCR complex, anti-CD3 mAb. This allows one to distinguish events that require ligation of the TCR from those that do not. Our results indicate that ligation of the TCR, in this case via FcR and anti-CD3, is not required for dendritic cells to bind T cells, but that presentation to the TCR is essential for subsequent entry into cell cycle (Fig. 4, Table I). Further experiments will be required to compare the rate of APC-T cell binding in the presence and absence of anti-CD3, but for the time being, it is evident that dendritic cells do not need to be presenting a ligand to the TCR to bind resting lymphocytes in large numbers. In contrast, macrophages require FcR and anti-CD3 to bind these same T cells (Fig. 4).

Antigen-independent Clustering as a Feature of Dendritic Cell Function. In addition to the studies reported here, other lines of evidence have indicated that dendritic cells have the capacity to temporarily bind to T lymphocytes, and that this binding precedes the delivery of a signal to the TCR complex. The first observations involved stimulated T lymphoblasts. The latter were generated to one set of antigens and then shown to bind to dendritic cells that did not carry these antigens (7, 29). In ongoing experiments, continuous video monitoring is showing that dendritic cells move about the culture vessel constantly surveying and binding T cells before some are retained.

The binding mechanism is not yet identified. Many mAbs to accessory molecules like CD4 and LFA-1 do not block dendritic-T cell clustering but block T cell function after clustering has occurred (30).

In contrast to dendritic cells, macrophage binding of T cells requires FcR and anti-CD3 (Fig. 4). Macrophages also fail to bind resting antigen-specific lymphocytes in a primary MLR (6, 30, 31), but do bind to lymphoblasts in an antigen-dependent fashion once they have been sensitized by dendritic cells (7, 31). It is possible that APC other than dendritic cells can be induced to bind resting T cells.

Recently, this has been demonstrated for B blasts that have been induced with anti-Ig (32).

In this paper, we also compared the capacity of epidermal LC to form stable clusters with T cells in the anti-CD3 system. We found that freshly isolated LC could form loose T cell aggregates in the presence of anti-CD3, but that the clusters quickly fell apart and did not exhibit DNA synthesis (Table II). Interestingly, freshly isolated LC have 10 times more FcγRII than cultured LC (12), yet the former only weakly retain and stimulate T cells. This defect is not due to the use of trypsin to prepare the LC, since trypsinization of cultured LC does not block function (27). The upregulation of the dendritic cell binding mechanism may be an early step in the immune response, allowing this APC to sample and retain those T cell clones that recognize the specific peptides presented on the dendritic cell surface.

Role of Lymphocyte-activating Factors in the Function of Dendritic Cells. Only a brief period (8 h or less) of contact with dendritic cells seems to be required before some of the T cells can synthesize DNA in response to IL-2 (Table IV). This tempo of acquisition of IL-2 responsiveness is comparable with that previously found using sodium periodate as a mitogen (33).

While IL-2 can drive DNA synthesis and division in T cells that have interacted with dendritic cells, it remains to be established if so-called lymphocyte-activating factors are required at an earlier stage of mitogenesis. To date, the production of one candidate activating factor, IL-1, has not been detected (34–36). One wonders if other activating factors might be present. To look for such factors we cultured interacting dendritic cells and T lymphocytes across a filter from T cells, with both populations being exposed to anti-CD3 mitogen (Tables III and IV) or transplantation antigen (not shown). To our knowledge, such two-chamber experiments have not been used to look for lymphocyte-activating factors. We found that T cells did not proliferate even when coated with anti-CD3 and bathed in IL-2 derived from the clusters. This indicates that neither accessory cells, nor T cells interacting with dendritic cells, release activating factors that directly induce growth or responsiveness to growth factors. Conceivably small amounts of an essential factor are released locally in the contact zones between dendritic cells and T lymphocytes. Alternatively, antigen-independent binding to dendritic cells followed by signaling the TCR may be the two key events in initiating lymphokine synthesis and responsiveness during T cell responses.

Summary

Dendritic cells bearing antigen efficiently aggregate and stimulate antigen-specific T cells. We describe an experimental model in which an initial, apparently antigen-independent binding step is followed by ligation of the TCR. The model is the polyclonal response to mAb to the CD3 portion of the TCR complex. Epidermal and thymic dendritic cells utilize low levels of Fc receptors to present the anti-CD3 mAb and induce mitogenesis. Within 3 h of coculture, most of the dendritic cells have formed clusters with the resting T lymphocytes, and these clusters are the site for subsequent DNA synthesis and cell growth. However, the binding of dendritic cells to T cells proceeds as efficiently in the absence of anti-CD3 as in its presence, and anti-FcR mAb does not block. CD3 and Fc receptors are essential for the subsequent

mitogenesis response in dendritic-T cell clusters. Because an exogenous ligand for the TCR does not seem to be required for the extensive polyclonal clustering of resting lymphocytes to dendritic cells, we suggest that an antigen-independent mechanism mediates the initial interaction. This clustering seems essential for T cell growth since we do not detect, in two-chamber experiments, soluble lymphocyte-activating factors that originate from dendritic-T cell aggregates and that activate anti-CD3-coated T cells.

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