Dendritic Cells Pulsed with Protein Antigens In Vitro Can Prime Antigen-specific, MHC-restricted T Cells In Situ

By Kayo Inaba, Joshua P. Metlay, Mary T. Crowley, and Ralph M. Steinman

From The Rockefeller University and Irvington Institute, New York, New York 10021

Summary

T cells recognize peptides that are bound to MHC molecules on the surface of different types of antigen-presenting cells (APC). Antigen presentation most often is studied using T cells that have undergone priming in situ, or cell lines that have been chronically stimulated in vitro. The use of primed cells provides sufficient numbers of antigen-reactive lymphocytes for experimental study. A more complete understanding of immunogenicity, however, requires that one develop systems for studying the onset of a T cell response from unprimed lymphocytes, especially in situ. Here it is shown that mouse T cells can be reliably primed in situ using dendritic cells as APC. The dendritic cells were isolated from spleen, pulsed with protein antigens, and then administered to naive mice. Antigen-responsive T cells developed in the draining lymphoid tissue, and these T cells only recognized protein when presented on cells bearing the same MHC products as the original priming dendritic cells. In contrast, little or no priming was seen if antigen-pulsed spleen cells or peritoneal cells were injected. Since very small amounts of the foreign protein were visualized within endocytic vacuoles of antigen-pulsed dendritic cells, it is suggested that dendritic cells have a small but relevant vacuolar system for presenting antigens over a several day period in situ.

The immunologic activity of T lymphocytes is directed to antigens presented by MHC products on the surfaces of other cells termed APC (1–4). While many cell types are capable of generating MHC–peptide complexes and presenting these to primed T cells, it is evident that the dendritic cell subset of APC greatly accelerates the early sensitization phase of the immune response. This has been noted in vitro with transplantation (5–7) and viral (8, 9) antigens, and in situ using contact (10, 11) and transplantation antigens (12–14). Nevertheless, experimental studies of T cell sensitization in situ to antigens that require processing typically utilize artificial adjuvants rather than viable APC. Whenever bulk spleen cells have been used as APC in the absence of adjuvants, it has not been possible to restrict the sensitization to antigens in association with MHC products of the injected cells (15, 16). Therefore presentation in situ likely involves host rather than injected APC.

Kurt-Jones et al. used B cells as APC in situ to reverse a lack of T-cell responsiveness in mice that had been suppressed chronically with anti-μ antiserum (17). The B cells, when given simultaneously with antigen in CFA, appeared to be capable of priming MHC-restricted T cells in some but not all cases. In contrast, Lassila et al. (18) reported that in a chicken system B cells could not present antigens to T cells in situ. Likewise, in primary antibody responses to hapten-carrier conjugates in vitro, dendritic cells and not B cells are required as APC early in the immune response (19).

Here we have assessed the capacity of antigen-pulsed dendritic cells to sensitize the T cells of an unprimed individual. We find that specific priming occurs, and that the sensitized T cells are restricted to recognize antigen on the MHC products of the presenting dendritic cells. These results suggest that dendritic cells are "nature's adjuvant." They are capable of delivering exogenous antigens, most likely as complexes of peptides on surface MHC products, directly to naive T cells in situ.

Materials and Methods

Mice. BALB/C × DBA/2 (CxD2)F_1 (H-2^d), C3H/HeJ (H-2^k), and (C3H × DBA/2)F_1 mice, 6–12 wk old and of both sexes, were purchased from The Trudeau Institute, Saranac Lake, NY.

Proteins. The antigens tested were spermwhale myoglobin, conalbumin, human gamma globulin, ovalbumin (Sigma Chemical Co., St. Louis, MO) and rhodamine-modified ovalbumin (Molecular Probes, Eugene, OR).

Culture Medium. For the preparation and antigen-pulsing of dendritic cells, the medium was RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 5% FCS, 50 μM 2-ME,

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and 20 μg/ml gentamicin. For assessing T cell proliferative responses in vitro, the medium was Click's medium (Irvine Scientific, Santa Ana, CA) supplemented with 0.5% heat-inactivated mouse serum, 50 μM 2-ME, and 20 μg/ml gentamicin.

Antigen "Pulsing" of APC. As will be evident in Results, it was necessary to expose fresh rather than cultured dendritic cells to a foreign protein to successfully charge these APC with antigen. Adherent cells from a low buoyant density fraction of spleen were prepared (20–22) and cultured overnight (12–18 h) in medium to which 0.1 mg/ml of protein antigen was added. After overnight culture, the dendritic cells were purified by rosetting most of the contaminants (macrophages, B cells) with antibody-coated erythrocytes (21, 22). In experiments to be reported elsewhere (23), we have found that fresh dendritic cells purified on a FACS can be charged with antigen with just a 3-h exposure. However, the longer overnight "pulse" (12–18 h) was used, since it simplified the purification of the dendritic cells and improved their APC function. To ensure that the dendritic cells had been successfully pulsed with antigen, we did conventional restimulation assays using primed lymph node T cells (see Results). The other APC populations that were pulsed with foreign proteins were resident peritoneal cells, maintained in Teflon beakers to reduce macrophage adherence, and unfractionated spleen cells. In some experiments, 4 mg of protein was given to mice intravenously or intraperitoneally, spleen dendritic cells were isolated as described (24), and these in vivo pulsed APC were readministered to naive mice.

Priming with Antigen-pulsed APC In Situ. Antigen-pulsed APC were washed at least three times in RPMI 1640 and administered in PBS at a dose of 2–60 × 10^5 cells in a volume of 25–40 μl into the fore or hind footpads. Generally, the antigen-pulsed APC were administered on one side, and the contralateral footpads served as the control. The control footpads were injected with APC that either had not been antigen-pulsed or were pulsed with a noncross-reacting protein (see Results). At varying times thereafter, but usually at day 5, the draining popliteal or brachial lymph nodes were removed, teased into a cell suspension, and challenged with antigen in vitro at 1–100 μg/ml. 3 × 10^5 cells were cultured in triplicate in flat-bottomed microtest wells (No. 25860; Corning Glassworks, Corning, NY). DNA synthesis was measured on the 3rd day after exposure to [^{3}H]TdR (specific activity, 6.0 Ci/mM) at 4 μCi/ml for 12–16 h. Unprimed lymph nodes never showed a response to the antigens we studied. The cells that responded by DNA synthesis in primed mice were shown to be primarily CD4$^+$ Thy-1$^+$ cells by treatment with appropriate mAb and complement before the assay for DNA synthesis.

MHC Restriction of In Vivo Primed T Cells. C3H × DBA/2 or A × DBA/2 F_1 (Ia^k × Ia^d) mice were primed with antigen-pulsed dendritic cells from either parental strain. 5 d later the draining lymph nodes were taken, and the cell suspensions were treated with mAb J11d anti-B cell and dendritic cell (22) and B21-2 anti-I-A plus rabbit complement (Pel-Freeze Biologicals, Rogers, AR) to deplete lymph node APC. The cells were then cultured at 3 × 10^5 cells per microtest well in triplicate with graded doses of irradiated (1,000 rad ^{137}Cs) parental or F_1 spleen cells as APC with or without antigen. As will be evident in the Results, responsiveness was observed primarily when antigen was presented by the same parent that was used to prime the animals. To verify that the response was class II MHC-restricted, blocking studies with culture supernatants of anti-Ia mAbs were performed. The mAbs were B21-2 anti-Ia^d and 10-2.16 anti-Ia^k, both available at the American Type Culture Collection, Rockville, MD (TIB 229 and TIB 93, respectively).

Pinocytosis of Protein Antigens. Rhodamine-modified ovalbumin proved to be a sensitive protein for visualizing pinocytosis by the weakly endocytic dendritic cells. Uptake was apparent after an overnight exposure to 0.1 mg/ml. Little or no uptake was evident at 0.02 mg/ml, or after a 2-h exposure, using fluorescence microscopy with a Zeiss Axiomat equipped for epifluorescence. The other tracers which we tested, which showed less and sometimes no uptake at the light microscopic level, were FITC-dextran, lucifer yellow, and horseradish peroxidase all at 0.1 mg/ml, the dose used to charge the APC with antigen. The positive control for active pinocytic activity was provided by resident macrophages in peritoneal washouts (see Results).

Results

Conditions for Pulsing Mouse Dendritic Cells with Protein Antigens In Vitro. We began with sperm whale myoglobulin, for which prior studies had defined an immunodominant region in the H-2^d mouse corresponding to residues 106–118 (25, 26). Spleen adherent cells, which include dendritic cells, macrophages, and B cells, were cultured with or without native myoglobin overnight (16–24 h). The dendritic cells were then enriched by a standard method (21, 22) and tested for their capacity to stimulate myoglobin-primed T cells. As shown before (27), dendritic cells that had been cultured overnight without antigen were able to present peptide fragments, but presented native protein only weakly (Table 1, compare group 3 with groups 1 and 2). However, if the dendritic cells had been exposed to protein during the overnight culture, the antigen-pulsed APC vigorously stimulated the primed T cells [Table 1, group 4]. Similar findings were made with other proteins (human gamma globulin, conalbumin, and ovalbumin), but we did not have active peptide fragments for these antigens. Similar results also were obtained if the dendritic cells were cultured for 2 d before use (Table 1, compare group 7 with groups 5 and 6). However, it was noted that the antigen pulse was best if given during the first rather than the second day of culture (Table 1, compare groups 8–10). Once pulsed, the dendritic cell maintained immunogenicity for at least a day in culture (Table 1, group 10). We conclude that freshly isolated dendritic cells can be successfully pulsed with a variety of soluble protein antigens in vitro, but that it is important to administer the antigen shortly after isolating the dendritic cells from the spleen.

Antigen-pulsed Dendritic Cells Sensitize T Cells In Situ. After exposure to one of four different proteins, as above, the dendritic cells were injected into the left hind foot pad; companion unpulsed dendritic cells were injected into the right side. The draining popliteal lymph nodes from groups of three to five mice were taken 5 d later and tested for responses in vitro to each of three different proteins. To avoid responses to FCS components, which were present during the time that the dendritic cells were pulsed with antigen, the lymph node cells were cultured in the presence of mouse rather than fetal calf serum.

For each protein, the lymph node draining the site of antigen-pulsed, dendritic cell deposition developed specific antigen responsiveness (Table 2). If we injected two populations of dendritic cells, each pulsed with different proteins, then the lymph node cells acquired reactivity to both antigens.

Table 1. *Conditions for Pulsing Dendritic Cells with a Foreign Protein In Vitro*

Group	Culture of dendritic cells before use as APC*	Antigen‡ during the APC-T coculture	DNA synthesis by antigen-primed T cells§ to graded doses of dendritic cells‖			
			3×10^4	10^4	3×10^3	10^3
1	0–24 h, no antigen	None	3.9	1.9	0.8	0.2
2	0–24 h, no antigen	Myoglobin	7.6	1.6	0.5	0.2
3	0–24 h, no antigen	Myopeptide	45.9	20.2	6.9	1.2
4	0–24 h, myoglobin	None	91.6	37.9	10.8	4.2
5	0–48 h, no antigen	None	3.6	1.4	0.7	0.3
6	0–48 h, no antigen	Myoglobin	0.3	0.6	0.3	0.3
7	0–48 h, no antigen	Myopeptide	39.2	18.2	4.7	0.7
8	0–48 h, myoglobin	None	81.8	27.3	8.9	3.2
9	0–24 without myo, 24–48 with myo	None	15.0	4.4	1.7	0.7
10	0–24 with myo, 24–48 without myo	None	88.5	27.6	9.8	4.0

* Low density spleen adherent cells, which are a partially enriched population of dendritic cells (20–22), were cultured for 1 or 2 d in medium supplemented with antigen (100 μg/ml sperm whale myoglobin; Fluka) where indicated. After culture, contaminating macrophages and B cells were removed by rosetting with antibody-coated red cells.

‡ No antigen, myoglobin (5 μM), or myoglobin peptide 105–118 (2.5 μM), was added to the coculture of antigen-pulsed dendritic cells and myoglobin-primed T cells (below).

§ Antigen-primed T cells were prepared as follows. Mice were primed with 5 μm of myoglobin in CFA in the footpads. Brachial and/or popliteal lymph nodes were taken 5 d later and cell suspensions were prepared by teasing the nodes with forceps. The cell suspensions were cultured at 5×10^6 cells/well in 24-well trays in 1.5 ml Click's medium supplemented with 0.5% mouse serum, 2 mM L-glutamine, 50 μM 2-ME, and 5 μM myoglobin. 10 d later the contents of the flask were applied to Ficoll (Sigma) columns to collect viable lymphoblasts. By using T cells that had been expanded in vitro, rather than fresh lymph node cells, we could obtain populations that responded strongly to restimulation with specific antigen on dendritic cells, without the syngeneic mixed leukocyte reaction that typically elevates background DNA synthesis when lymph node cells are cultured with unpulsed dendritic cells.

‖ 3×10^4 myoglobin-primed T blasts (see §) were cultured with graded doses of dendritic cells (see *) [^{3}H]TdR was added at 48–64 h to measure DNA synthesis. Data are mean cpm [^{3}H]TdR uptake $\times 10^{-3}$ for triplicate wells; standard deviations were <10% of the mean. The experiment was repeated once with similar results.

In these latter experiments, there was some development of antigen reactivity in the nondraining lymph node (Table 2, last pair of antigens).

In experiments that are not shown, the antigen-reactive lymph node cells were CD4$^+$ T cells primarily, since >80% of the reactivity could be eliminated with either anti-Thy-1 or anti-CD4 mAb and complement. The sensitizing capacity of dendritic cells was reproducible, in that individual mice each gave responses of comparable magnitude.

In kinetic studies, the draining lymph node cells became responsive over a 5-d period to the specific protein that had been used to pulse the injected dendritic cells (Fig. 1). Antigen specificity was maintained at all time points, i.e., if the left foot pad had been injected with dendritic cells pulsed with human gamma globulin, the left popliteal node developed specific responsiveness to human gamma globulin but not to other proteins (Fig. 1, *left*). Likewise, the right popliteal node developed responsiveness to the protein used to pulse the dendritic cells that were injected into the right food pad (Fig. 1, *right*).

When primed mice were rechallenged with antigen-pulsed dendritic cells but in a site distal to that used for the original priming (front vs. hind foot pad), the lymph node draining this second site showed an accelerated or "memory" type response to the appropriately pulsed dendritic cells (Fig. 2). Responsiveness to antigen was apparent on the second day and virtually disappeared by the third.

Dendritic cells were also charged with a 2-h pulse of protein antigen in vivo as recently described (24). The dendritic cells were purified from the spleen using the FACS and injected into naive mice. Specific priming to the protein that originally had been given systemically to the dendritic cell donor was then observed (Table 3). We conclude that dendritic cells that had been pulsed with protein antigen in vitro or in vivo are capable of sensitizing CD4$^+$ T cells from naive mice to that protein.

APC Requirements for Successful Priming In Situ. Dendritic cells were compared with two standard populations that have been used in many studies of antigen presentation in vitro. These were suspensions from spleen (a rich source of B lymphocytes) and peritoneal cavity (a rich source of macrophages and CD5$^+$ B cells). The populations were pulsed with antigen for either 3 h or for 18 h in culture and administered in graded doses of 8×10^5 to 5×10^6 cells to the footpads of naive recipients (Table 4). Spleen cells were marginally effective, in that only the highest dose of 6×10^6 cells induced

Table 2. *Dendritic Cells that Are Pulsed with a Protein Antigen In Vitro Specifically Prime Animals to that Protein In Situ*

Popliteal node	DC pulsed with:	DNA synthesis after challenge with						
			Conalbumin		Myoglobin		Hu × gamma globulin	
		No Ag	100	10	100	10	100	10
					cpm × 10^{-3}			
Right	No Ag	0.4	0.7	0.4	0.7	0.6	3.2	2.0
Left	Conalbumin	0.3	126.1	92.7	1.3	0.8	3.4	0.9
Right	Myoglobin	0.2	0.4	0.2	32.3	33.9	1.4	0.9
Left	No Ag	0.2	0.5	0.3	0.3	0.4	1.9	0.1
Right	HGG	0.2	0.5	0.3	0.2	0.4	27.4	18.0
Left	No Ag	0.4	0.8	0.7	0.7	0.4	2.6	2.3
Right	Conalbumin + Myoglobin	0.5	58.7	18.5	40.0	42.7	3.7	2.4
Left	HGG	0.2	0.3	0.2	0.3	0.3	18.6	11.6
Right	Conalbumin	0.7	99.6	79.5	2.2	1.2	19.6	8.7
Left	Myoglobin + HGG	0.7	8.7	4.5	73.8	62.9	119.4	104.6
Right/left	No DC	0.1	0.2	0.2	0.2	0.2	0.6	0.3

Low density spleen adherent cells were cultured for 1 d with or without antigen, after which macrophages and B cells were depleted (21, 22). 2 × 10^5 dendritic cells were injected in the foot pads of groups of four mice. 5 d later, the draining popliteal nodes were taken and cell suspensions were prepared by teasing with fine forceps. 3 × 10^5 primed lymph node cells were cultured in flat-bottomed microtiter wells without antigen, or with the indicated antigens at 100 or 10 μg/ml. [^{3}H]TdR was added at 44–60 h to measure DNA synthesis.

Table 3. *Priming of Antigen Specific Lymph Node T Cells Using Dendritic Cells that Have Been Pulsed with Antigen In Vitro or In Vivo*

Dendritic cells [2 × 10^5] used to prime brachial nodes	DNA synthesis of primed lymph node boosted in vitro with*					
		Conalbumin (μg/ml)			BSA	OVA
	No Ag	100	10	1	100	100
				cpm × 10^{-3}		
In vitro pulse‡						
Myoglobin	0.1	0.1	—	—	0.9	—
Conalbumin	0.4	155.4	148.8	119.3	3.3	1.1
Conalbumin pulse + 25 μg soluble Ag in paw	0.4	161.8	145.3	124.1	4.8	0.3
In vivo§						
Conalbumin	0.3	68.7	45.5	22.1	1.5	1.0

* 5 d after priming with dendritic cells in the front footpad, brachial lymph node cells were prepared and restimulated in culture with the indicated antigen. DNA synthesis was measured on the third day.

‡ Low density spleen adherent cells were pulsed with 100 μg/ml of protein overnight. Dendritic cells were then purified by depleting FcR$^+$ cells (21, 22).

§ Mice were given 4 mg conalbumin i.v. 2 h later, the spleens were taken, and dendritic cells were isolated using the FACS and the N418 mAb to murine CD11c (24). N418 primarily reacts with dendritic cells in mouse spleen (23).

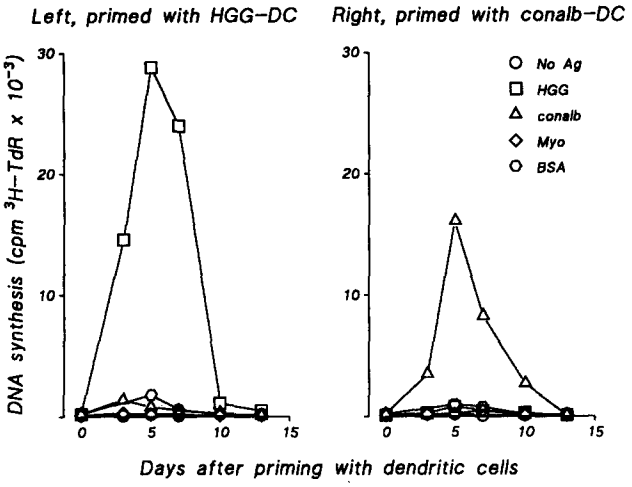

Figure 1. Kinetics of the primary response to antigen-pulsed dendritic cells. Low density spleen adherent cells were cultured with or without 100 μg/ml of the indicated proteins for 24 h. The cells were washed and 3 × 10^5, FcR$^-$ dendritic cells were injected into each foot pad. At the indicated time points, the draining popliteal and brachial lymph nodes were taken and cultured with the indicated proteins at 100 μg/ml in Click's medium supplemented with 0.5% mouse serum. DNA synthesis was measured on the third day. Lymph node cells that were primed with dendritic cells that had not been pulsed with antigen did not exhibit an antigen response (not shown, but see Table 2). The experiments were repeated twice with similar results.

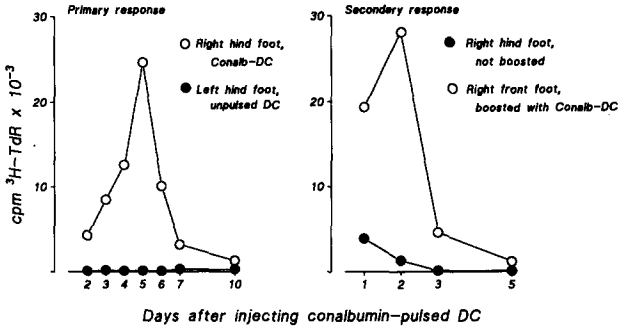

Figure 2. Secondary responses in mice that had been primed with antigen-pulsed dendritic cells. Conalbumin-pulsed dendritic cells (*DC*) were injected into the right hind foot pad as in Fig. 1, while the left hind foot pad received dendritic cells that were not pulsed with conalbumin. At the indicated time points (*left*), the draining lymph nodes were taken and boosted with protein antigen in vitro. Note that the primary response peaks at day 5 and subsides by day 7–9, as in Fig. 1. Companion groups of mice were then boosted on day 12 with antigen-pulsed DC, but in the right front foot pad to look for a secondary response (*right*). The experiment was repeated twice with similar results.

a low level of responsiveness in the draining lymph node. Peritoneal cells were ineffective at all doses. Inocula of 2 and 5 × 10^5 dendritic cells had similar effects in situ, and the minimum dose capable of inducing some responsiveness was 3–8 × 10^4 dendritic cells (Table 4).

MHC Restriction of T Cells Primed by Antigen-pulsed Dendritic Cells. The finding that antigen-pulsed dendritic cells could prime naive animals to that antigen could be explained by a unique ability of dendritic cells to stimulate T cells directly in vivo, or alternatively, to transport antigens that were presented subsequently by host APC. The two possibilities could be distinguished by assessing whether the T cell sensitization process was restricted to antigens presented on the injected vs. host dendritic cells. We primed F_1 mice with antigen-pulsed dendritic cells from either parental strain and tested if the primed F_1 T cells could only be boosted with spleen APC from the original parent. It is known that most clones of T lymphocytes in an F_1 animal are restricted to antigens presented by one or the other parental MHC (28, 29). We used AxDBA/2 (H-2^a × H-2^d) or C3HxDBA/2 (H-2^k × H-2^d) F_1 recipients and primed with antigen-pulsed dendritic cells from each parental strain. 5 d later the F_1 lymph node cells were isolated, depleted of endogenous APC by treatment with anti-Ia and J11d mAb and complement, and challenged with APC from the F_1 or from either parent.

The F_1 T cells responded vigorously to antigen rechallenge in vitro with F_1 APC (Table 5). If parental strain APC were used, the rechallenge was far more effective with APC from the same parental strain that was used to sensitive the local node (Table 5). To show that the responses were restricted to the class II MHC molecules of the sensitizing dendritic cells, we verified that a mAb to I-A^d blocked the restimulation of F_1 T cells primed with H-2^d APC but not with H-2^a APC, while mAb to I-A^k blocked presentation by H-2^k APC but not H-2^d APC (Table 6).

Visualization of Antigen Uptake in Dendritic Cells. Since one pathway for antigen presentation likely involves endocytosis of the foreign protein followed by proteolysis and formation of peptide-MHC complexes (4), we monitored the extent to which dendritic cells could accumulate a protein that we could visualize, rhodamine-modified ovalbumin. When dendritic cells were pulsed overnight in 0.1 mg/ml of protein, it was evident that each cell had a small number of fluorescent granules, usually close to the nucleus (Fig. 3, *left*). Macrophages in contrast were much more heavily labeled after exposure to rhodamine-ovalbumin (Fig. 3, *right*). These results suggest that the strong APC function of dendritic cells in situ is associated with the accumulation of only small amounts of the foreign protein.

To verify that the rhodamine tracer that was being visualized in the above experiments was in fact relevant to immunogenicity in situ, we charged dendritic cells with rhodamine-modified ovalbumin and administered the cells to mice. 5 d later cells from the draining lymph node were tested for antigen responsiveness. Interestingly, responsiveness developed in a dose-dependent manner (Fig. 4, *left*) but it was specific for the conjugate rather than the free ovalbumin carrier (Fig. 4, *right*). Therefore, the rhodamine group that we were monitoring in the uptake studies above (Fig. 3) was relevant to the antigen-specific sensitization that was occurring in situ.

Discussion

Several features of dendritic cells help explain the sensitizing function of these APC in situ (30). One is their capacity to

Table 4. *Dendritic Cells Are Specialized to Present Antigens In Vivo*

Exp.	Myoglobin-pulsed APC used to prime in vivo		Proliferation of primed lymph node cells with			
				Myoglobin		Conalbumin
	Cell type	Number of cells	No Antigen	5 μm	0.5 μm	100 μg/ml
1	Spleen cells	5×10^6	0.1	5.6	0.8	0.2
		2×10^6	0.1	2.1	0.2	0.2
		8×10^5	0.1	0.1	0.1	0.2
	Dendritic cells	5×10^5	0.1	32.5	28.1	0.3
		2×10^5	0.1	29.9	17.6	0.1
		8×10^4	0.1	4.2	0.6	0.1
	Peritonal cells	2×10^6	0.1	0.1	–	0.1
		8×10^5	0.1	0.1	0.1	0.1
	None	–	0.1	0.1	0.1	0.1

	Conalbumin-pulsed APC used to prime in vivo		Proliferation of primed lymph node cells with				
				Conalbumin (μg/ml)			Ovalbumin
	Cell type	Number of cells	No Antigen	100	10	1	100
2	Spleen cells	2×10^6	0.3	9.8	6.3	2.0	0.2
		1×10^6	0.3	2.1	0.5	0.2	0.2
		5×10^5	0.3	0.3	0.3	0.2	0.2
		2.5×10^5	0.3	0.3	0.2	0.2	0.2
	Dendritic cells	5×10^5	0.3	56.3	54.2	19.6	0.3
		2.5×10^5	0.3	47.2	40.1	12.1	0.3
	Peritonal cells	2×10^6	0.2	0.4	0.3	0.3	0.2
		1×10^6	0.2	0.3	0.3	0.2	0.2
		5×10^5	0.2	0.3	0.2	0.2	0.2
		2.5×10^5	0.2	0.2	0.2	0.2	0.2

Three different populations of APC were exposed to 100 μg/ml of myoglobin for 16 h (Exp. 1) or conalbumin (Exp. 2), washed, and administered into the footpads of naive mice at the indicated doses. 5 d later the draining lymph nodes were taken, and the cell suspensions were restimulated with antigens as shown. Data are cpm [^{3}H]TdR uptake $\times 10^{-3}$ at 48–60 h. Data are not shown for APC that were *not* pulsed with antigen before administration to mice, since the proliferative responses were all 0.3 or less.

capture and retain antigens, a feature that seems to be short-lived in culture (Table 1) (27). Another is their capacity to form stable conjugates with resting, antigen-specific T cells and induce the development of functional T lymphoblasts (31–33). A third feature is the ability to home via the lymph and the blood (34–36) to the T-dependent areas of lymphoid organs. These functions together should allow the antigen-pulsed dendritic cell to select clones of specific T cells from the recirculating pool (37).

The number of dendritic cells that were effective in our experiments, $1–3 \times 10^5$, is not large when other variables are considered. The efficiency with which these cells leave the injection site and home to the draining lymph node may be very small, since Kupiec-Weglinski et al. (34) reported that only 1–2% of a dose of ^{111}In-labeled dendritic cells were retained within the draining lymph node. This means that a few thousand dendritic cells may carry out the sensitization of T cells reported here. A similar conclusion was reached in studies of pancreatic islet transplantation. There, a maximum of 2,000–4,000 dendritic cells seemed responsible for sensitizing mice across an MHC barrier (13). The efficiency of these APCs likely relates to the fact that they home to

Table 5. *Primed F_1 T Cells Are Restricted to the Parental Strain of the Dendritic Cells Used in Priming*

Exp.	Spleen APC used to boost F_1 T cells *in vitro*		DNA synthesis by F_1 T cells			
			Primed with Ia^k DC		Primed with Ia^d DC	
	Strain	Dose	−Ag	+Ag	−Ag	+Ag
			cpm × 10^{-3}			
A: A × DBA/2 F_1 T cells	A × DBA/2 [Ia^{kxd}]	3 × 10^5	0.6	11.4	0.3	11.8
	A	3 × 10^5	0.5	22.4	0.1	0.1
	[Ia^k]	5 × 10^5	0.8	29.6	0.3	0.6
	BALB/C	3 × 10^5	0.3	2.7	0.2	22.9
	× DBA/2	5 × 10^5	0.4	3.6	0.6	65.4
	[Ia^{dxd}]					
B: C3H × DBA/2 F_1 T cells	C3H × DBA/2	1.25 × 10^5	0.2	14.7	0.3	58.2
	[Ia^{kxd}]	2.50 × 10^5	0.6	21.8	0.5	73.0
		5 × 10^5	1.1	25.0	3.4	67.1
	BALB/C	1.25 × 10^5	0.2	0.5	0.2	46.7
	× DBA/2	2.50 × 10^5	0.8	1.3	0.9	76.7
	[Ia^{dxd}]	5 × 10^5	2.0	2.2	2.5	69.0
	C3H	1.25 × 10^5	0.1	13.3	0.4	0.8
	[Ia^k]	2.50 × 10^5	0.4	31.6	1.2	1.4
		5 × 10^5	1.8	26.2	2.9	3.4

F_1 mice (Ia^{kxd}) were primed with 3 × 10^5 dendritic cells (DC) that had been pulsed with 100 μg/ml of conalbumin. The dendritic cells were from either parental strain. 5 d later, the lymph nodes were taken and cell suspensions were prepared by teasing with fine forceps. These suspensions were treated with B21-2 anti-Ia, and J11d mAb and complement to deplete endogenous APC, and then plated at 3 × 10^5 cells in flat-bottomed microtiter wells in Click's medium with 0.5% mouse serum. Irradiated spleen cells (1,500 rad ^{137}Cs) from either parental strain, or the F_1 strain, were then added at the indicated doses as a source of APC. Conalbumin was (+Ag) or was not (−Ag) added to the cultures at 100 μg/ml. DNA synthesis was measured at 44–60 h.

Table 6. *Priming by Antigen-pulsed Dendritic Cells In Vivo is MHC-restricted*

Strain of dendritic cells used to prime C3H × D2 F_1 T cells*	Strain of spleen used to present antigen in vitro‡	DNA synthesis in presence of:§			
		no Ab	αCD4	αIa^k	αIa^d
		cpm × 10^{-3}			
C3H, H-2^k	C3H, H-2^k	55.9	8.5	11.7	42.6
	C3H, H-2^k	12.5	1.1	3.2	10.1
BALB/C × DBA/2, H-2^d	BALB/C × DBA/2, H-2^d	155.5	12.0	128.0	23.8
	C3H × DBA/2 (H-2^{kxd})	86.2	9.0	41.7	12.9

* 2 × 10^5 dendritic cells, pulsed with conalbumin, were injected into C3H × DBA/2 F_1 [H-2^k × H-2^d] mice. 5 d later, lymph nodes were treated with antibodies to Ia and J11d + complement to deplete endogenous APC. 3 × 10^5 T cells were then cultured per well. The experiment was repeated once with similar results.

‡ 3 × 10^5 spleen cells from the indicated strains were irradiated (^{137}Cs, 1,000 rad) and added to the primed T cells.

§ DNA synthesis was measured by adding [^{3}H]TdR at 66–72 h. Conalbumin was added in all the experimental cultures that are shown, since proliferation in the absence of antigen was <1 × 10^3 cpm. The mAbs tested for blocking activity were GK1.5 αCD4, 10-12.16 αI-A^k, and B21-2 αI-A^d.

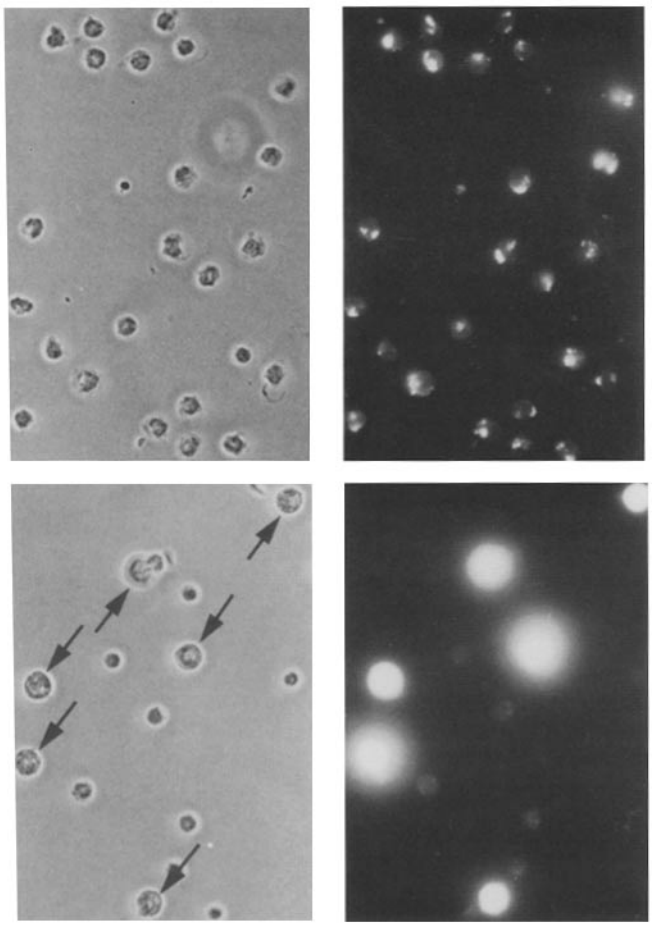

Figure 3. Detection of endocytic activity by antigen-pulsed dendritic cells. Low density spleen adherent cells were cultured overnight in 0.1 mg/ml of rhodamine-modified ovalbumin. The FcR^- dendritic cells were enriched and attached to glass slides coated with poly-L-lysine (21). The dendritic cells (*top*) were uniform in cytologic appearance (*left*) and each contained small numbers of rhodamine-labeled granules. In parallel, peritoneal cells were also exposed RITC-OVA. The large macrophages (*bottom, arrows*), as verified by strong indirect immunofluorescent staining with mAb to the M1/70 CD11b antigen, were intensely labeled with the endocytic tracer (*right*). ×300.

an optimal site in the lymphoid tissue and are each capable of binding and activating large numbers of T cells even with relatively small amounts of ligand on the dendritic cell surface (38).

Since the endocytic apparatus may provide an important route for the processing and presentation of exogenous antigens (4), it is of interest that the poorly endocytic dendritic cell (Fig. 3) is nonetheless extremely active in antigen presentation. This suggests to us that the endocytic apparatus of dendritic cells may be specialized to present antigens, whereas in macrophages, the bulk of the endocytic activity results in antigen clearance and degradation (39). In the experiments described here, it is formally possible that non-dendritic cells in the adherent spleen preparation were processing and regurgitating peptides onto dendritic cell MHC molecules, but in a recent study (24), we presented evidence that such a phenomenon is undetectable in our cultures. In addition, we have also prepared highly enriched populations of dendritic cells fresh from spleen, using the FACS, and found them to be potent APC for priming T cells in situ (23).

Once $CD4^+$ or $CD8^+$ T cells pass a control point that involves activation by dendritic cells, the sensitized T cells efficiently interact with other types of APC to carry out various effector functions that are critical for T cell–mediated immunity. For example, $CD4^+$ T blasts that are induced by dendritic cells can interact in an antigen-specific way to make B lymphocytes grow and respond to B cell stimulating factors (40), and to make macrophages synthesize IL-1 (41, 42). $CD8^+$ T blasts that are induced by dendritic cells can kill other APC as targets (43, 44). These "effector" aspects of the immune response may be restricted to inflammatory sites in situ, given the evidence that sensitized T cells that are produced in lymphoid tissues emerge into the lymph (45) and can move via the blood stream to inflammatory sites (46, 47). It is of interest that dendritic cells are not known to have any effector or antigen elimination functions, in contrast to other APC, such as B cells and macrophages which can re-

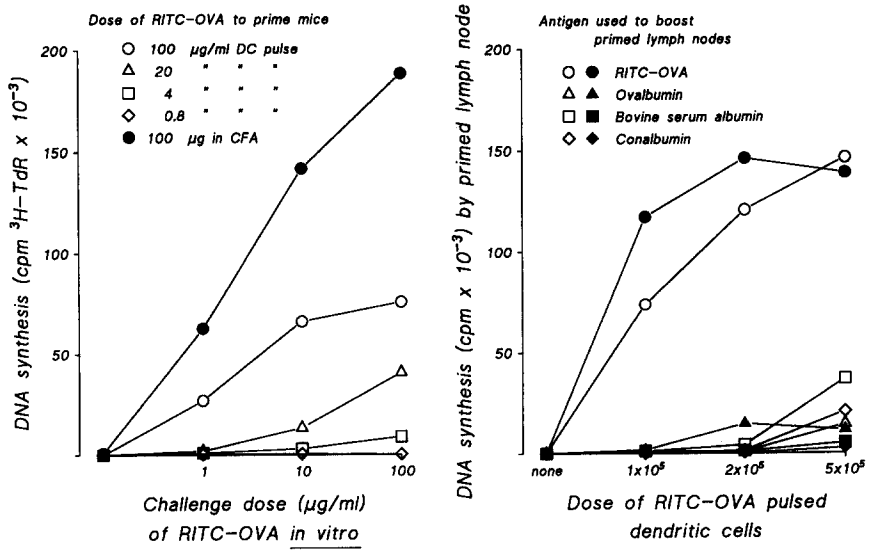

Figure 4. Rhodamine-ovalbumin (*RITC-OVA*)-pulsed dendritic cells prime mice in situ. Low density spleen adherent cells were exposed to graded doses of RITC-OVA (*left*) or to 0.1 mg/ml RITC-OVA (*right*) for 16 h, and then the dendritic cells were purified and injected at a dose of 3×10^5 into fore and hind foot pads (*left*) or in graded doses (*right*) into groups of three mice. 5 d later the draining lymph nodes were taken and boosted with graded doses of RITC-OVA (*left*) or RITC-OVA vs. other proteins (*right*). Only the data with 100 µg/ml protein are shown on the right, with the popliteal and brachial nodes in open and closed symbols, respectively. [³H]TdR uptake was measured at 48–60 h.

lease antibody or kill microorganisms. The specialized role of dendritic cells seems to be to sensitize T lymphoblasts, which then interact with other APC.

Extracorporeal pulsing of antigens onto dendritic cells may provide a new approach to immunization *in situ*, a goal that previously could be approached only empirically with adjuvants. By using dendritic cells as a natural adjuvant, one has an opportunity of having the APC select those epitopes on a complex antigen that can be presented by a given individual's MHC products. This strategy provides a physiologic selection of immunogen that may serve as an important alternative to the empirical search for immunogenic peptides that is being pursued so actively in recent years.

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Address correspondence to Dr. Ralph M. Steinman, The Rockefeller University Press, 1230 York Avenue, New York, NY 10021.

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