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Small Amounts of Superantigen, When Presented on Dendritic Cells, Are Sufficient to Initiate T Cell Responses

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Summary

Dendritic cells are potent antigen-presenting cells for several primary immune responses and therefore provide an opportunity for evaluating the amounts of cell-associated antigens that are required for inducing T cell–mediated immunity. Because dendritic cells express very high levels of major histocompatibility complex (MHC) class II products, it has been assumed that high levels of ligands bound to MHC products (“signal one”) are needed to stimulate quiescent T cells. Here we describe quantitative aspects underlying the stimulation of human blood T cells by a bacterial superantigen, staphylococcal enterotoxin A (SEA). The advantages of superantigens for quantitative studies of signal one are that these ligands: (a) engage MHC class II and the T cell receptor but do not require processing; (b) are efficiently presented to large numbers of quiescent T cells; and (c) can be pulsed onto dendritic cells before their application to T cells. Thus one can relate amounts of dendritic cell–associated SEA to subsequent lymphocyte stimulation. Using radiiodinated SEA, we noted that dendritic cells can bind 30–200 times more superantigen than B cells and monocytes. Nevertheless, this high SEA binding does not underlie the strong potency of dendritic cells to present antigen to T cells. Dendritic cells can sensitize quiescent T cells, isolated using monoclonals to appropriate CD45R epitopes, after a pulse of SEA that occupies a maximum of 0.1% of surface MHC class II molecules. This corresponds to an average of 2,000 molecules per dendritic cell. At these low doses of bound SEA, monoclonal antibodies to CD3, CD4, and CD28 almost completely block T cell proliferation. In addition to suggesting new roles for MHC class II on dendritic cells, especially the capture and retention of ligands at low external concentrations, the data reveal that primary T cells can generate a response to exceptionally low levels of signal one as long as these are delivered on dendritic cells.

During the initiation of an immune response, the antigen receptor–CD3 complex on T lymphocytes engages either peptide fragments or superantigens bound to MHC molecules on APCs, an event that is commonly referred to as “signal one.” To date there is little quantitative information on signal one using quiescent T cells. There are reports using cell lines as APCs and/or T cells (1, 2) where it has been found that only a few hundred MHC–peptide complexes are needed to stimulate IL-2 release from T-T hybridomas, for example. The affinity of these T cell lines may not be representative of bulk T cells, however, and the signal one requirements may be lower than quiescent T cells. The use of APCs and T cell lines may also obscure the contribution of accessory molecules that are thought to lead to the full complement of events that are needed during T cell growth and differentiation. Superantigens provide a means to pursue quantitative aspects of antigen presentation to human T cells. These molecules, many of them staphylococcal enterotoxins (SEs)1, resemble processed peptide antigens in the sense that they engage both the MHC class II molecules and the TCR (reviewed in reference 3). The experimental advantage of superantigens is that they do not require cellular processing (4, 5), so that one can better quantitate with trace-radiolabeled materials the amount of ligand associated with the APCs. Superantigen-pulsed APCs, particularly dendritic cells, stimulate responses in large numbers of quiescent T cells (6).

In this paper we use superantigens and dendritic cells in combination to gain information on the levels of signal one that are needed for primary T cell stimulation. Several re-

Abbreviation used in this paper: SE, staphylococcal enterotoxin.
sponses are initiated by antigen-bearing dendritic cells, including the induction of helper T cells for protein antigens (7, 8) and transplant rejection in situ (9, 10). Dendritic cells express very high levels of MHC class II, so that it has been tacitly assumed that the efficacy of these APCs relates to the higher amounts of MHC–peptide complexes that can be presented. Alternatively, function may be attributed to the presence on dendritic cells of several accessory molecules for T cell signaling (11). These hypotheses have been difficult to pursue directly, since it has not been possible to measure the levels of conventional antigenic peptides that are presented on MHC products by dendritic cells. Here we use radioiodinated SEA and human blood dendritic cells to gain such quantitative information. While blood dendritic cells, not surprisingly, bind much higher levels of SEA than other APCs, we find that very small numbers of superantigen–MHC class II complexes are sufficient to trigger resting primary T cells. At these low doses, triggering entails a major role of both CD4 and CD28 accessory molecules.

Materials and Methods

Culture Medium. RPMI 1640 (Gibco Laboratories, Grand Island, NY), supplemented with 5% human serum, 50 μg/ml gentamycin, and 10 mM Hepes was used throughout these studies except where indicated. Human sera were obtained from normal donors and were screened to ensure that they supported SEA-induced T cell proliferation at femtomolar concentrations. SEA used were similar to PCS in inducing responses to SEA (data not shown). Commercial sources of pooled human sera, however, have been problematic in supporting SEA-induced proliferation, presumably because of the presence of antistaphylococcal antibodies, and were not used here.

Preparation of Lymphoid Cells. Buffy coats were obtained from the New York Blood Center and separated into T cell–enriched (ER +) and T cell–depleted (ER −) fractions by rosetting with neuraminidase-treated SRBC.

T Cells. ER + cells were depleted of non-T cell contaminants first by panning on petri dishes coated with human gamma globulin to remove FeR + cells (12), and then coating MHC class II–positive cells with anti-DR/DQ mAb 9.3C9 (IgG2a, subclone of HB180; American Type Culture Collection, Rockville, MD) followed by panning on petri dishes coated with goat anti–mouse IgG (55459; Cappel Laboratories, Organon Teknika, Durham, NC [13, 14]). In some experiments T lymphocytes were prepared by passage of nonadherent ER + cells on nylon wool columns before panning with anti-MHC class II antibodies. The resulting T cell preparations failed to proliferate to optimal doses of superantigens, indicating negligible contamination with APCs.

CD4 + T Cells. These were enriched by negative selection of bulk T cells. The latter were incubated with αCD8 (OKT8, IgG2a, ATCC CRL 8014) and αCD11b (OKM1, IgG2b, ATCC CRL 8026) and panned on petri dishes coated with goat anti–mouse IgG. Naive and memory CD4 + subsets were negatively selected in a similar fashion by panning CD4 + T cells coated with αCD45RO (UCHL-1, IgG2a; gift of Dr. P.C.L. Beverley, Imperial Cancer Research Technology, London, UK) or αCD45RA (4G10, IgG2a), respectively. Cells depleted by panning constituted ≤2–3% of the resulting subpopulation as monitored by cytofluorography. The enrichment of CD4 + naive and memory T cell subsets was monitored by their relative responses to a crude extract of Candida albicans diluted 1:20 final in the presence of APCs.

APC Populations. To obtain monocytes, the ER − or T-depleted fraction was adhered to plastic for 60–90 min and subsequently dislodged by pipetting. The nonadherent non-T cells were subsequently used for purification of B cells and dendritic cells. Residual monocytes were first depleted from these populations by panning on gamma globulin–coated plates (12) and then layered onto 13–14 g metrizamide (15). After sedimentation at 650 g for 10 min, B cells and a few NK cells localized to the high-density pellet while dendritic cells were enriched in the low-density interface. The purity of dendritic cells ranged from 50 to 75% with contaminants being primarily small B and NK cells.

Lymphoblastoid Cell Lines. The Cox (MHC class II +) and Ket (BLS1, MHC class II −) cell lines were gifts of Dr. P. Cameron and Dr. J. S. Lee (16), respectively. They were maintained in RPMI supplemented with 10% FCS. We confirmed that the Ket cell lines lacked surface expression of class II molecules by staining with anti-MHC class II antibodies.

Mitogens. Highly purified SEA was purchased from Toxin Technology (Sarasota, FL). The mitogen was aliquoted to 1 mg/ml in sterile water, filtered, and stored at −20°C until use. SEA was confirmed to be >95% pure by SDS-PAGE and Coomassie staining.

Induction of SEA. SEA was iodinated using lactoperoxidase/glucose oxidase (Enzymobead Radioiodination Reagent; Bio-Rad Labs, Richmond, CA [17]). Specific activity ranged from 3.3 to 4.3 x 105 cpm/μg. The efficiency of iodination was 0.14 ± 0.04 mol of 125I/mol of SEA (n = 6 experiments). SEA was confirmed to be >95% pure by SDS-PAGE and autoradiography after iodination. Labeled and unlabeled SEA preparations were equally effective in stimulating T cells (not shown).

SEA Binding Assay. Aliquots of 105 to 2 x 106 of each APC type were incubated in 0.2 ml RPMI 1640/5% human serum and concentrations of 125I-SEA ranging from 0.015 to 150 nM for 1–1.5 h at 4°C or 37°C, in plastic microfuge tubes over a mixture of phthalate oils. At the termination of the assay, the tubes were spun at 8,000 g for 2 min, the tips containing cell pellets were cut off, and this portion ("bound") plus the cell-free component ("free") were counted in a gamma counter (18). Non-specific binding was determined from parallel tubes containing a 300-fold excess of unlabeled toxin.

Lymphocyte Proliferation Assays. APCs were added directly to cultures of T cells in the continuous presence of SEA in 96-well flat-bottomed plates (Costar, Cambridge, MA) in the continuous presence of 125I-labeled or nonlabeled SEA. The APC/T ratio ranged from 1:30 to 1:1,000. The APC/T ratio was determined from parallel tubes containing a 300-fold excess of unlabeled toxin.

Pulsed APCs. Graded doses of viable APCs were added to 105 purified T cells in 96-well flat-bottomed plates (Costar) in the continuous presence of SEA (nonpulsed APCs) or were first pulsed with SEA as described below (pulsed APCs).

Nonpulsed APCs. Graded doses of viable APCs were added to 105 purified T cells in 96-well flat-bottomed plates (Costar, Cambridge, MA) in the continuous presence of 125I-labeled or nonlabeled SEA. The APC/T ratio ranged from 1:30 to 1:1,000. The APC/T ratio was determined from parallel tubes containing a 300-fold excess of unlabeled toxin.
Anti-MHC Class II mAbs. Anti-MHC class II antibodies were used to block APC presentation of SEA to T cells. They consisted of L243 (anti-DR, IgG2a, ATCC HB55) and 9.3F10/subclone 9.3C9 (anti-DR/DQ, IgG2a, ATCC HB 180). Matched isotype controls were used at identical concentrations.

Assays to Evaluate the Contribution of Costimulator Molecules in Superantigen Presentation. 10⁶ T cells per flat-bottomed microwell were incubated at 4°C for 30 min with purified mAbs of the following specificities: αCD3 and αCD4 (Leu-4 and Leu-3a hybridomas, respectively, both IgG1; gift of Dr. R. Evans, New York, NY); αHLA DR/DQ (9.3F10/subclone 9.3C9, IgG2a, ATCC HB 180); and αCD28 (9.3, IgG2a; gift of Dr. E.A. Clark, Seattle, WA). Commercial murine IgG1 (MOPC 21) and IgG2a (UPC 10; Sigma Chemical Co., St. Louis, MO), dialedyzed free of azide, were used as isotype controls. Syngeneic dendritic cells were prepared as outlined above and “pulsed” with SEA (0.005 nM) for 1 h at 37°C before mixing with T cells at a responder/stimulator ratio of 100:1.

Results

SEA Binds in Large Amounts to MHC Class II Molecules on Dendritic Cells and with High Affinity. MHC class II molecules represent the major binding sites for superantigen on APCs (19–24). The number of class II binding sites on dendritic cells has not been evaluated in a quantitative manner with iodinated superantigens. We performed binding studies comparing dendritic cells to other primary populations of APCs such as B cells and monocytes. To quantify the amount of superantigen that can be bound, enriched populations of different APCs were exposed to iodinated SEA for 1–1.5 h at 4°C vs. 37°C. SEA was chosen since highly purified and stable preparations are available. Prior studies with toxic shock syndrome toxin 1 (TSST-1) and SEB have shown that equilibrium binding is usually complete within 1 h (22, 23).

Binding of SEA to dendritic cells was compared first to the class II—positive and —negative B cell lines Cox and Ket/BLS1 (the latter obtained from a patient with bare lymphocyte syndrome [16]). The binding characteristics of iodinated SEA to Cox, the class II—positive B cell line, and dendritic cells were similar. Saturable binding was evident (Fig. 1 A); and by Scatchard analysis, the data were consistent with a single receptor, most likely class II (Fig. 1 B). SEA bound to dendritic cells and Cox cells with high affinity (mean Kₐ values ranging from 5.6 to 13.4 nM) and to similar numbers of MHC class II molecules at saturation (average of 1.9–3.2 × 10⁶/cell; Table 1). It has previously been demonstrated that TSST-1 and SEB show saturable binding to APCs with Kₐ's in the range of 17–43 nM (19, 22) and 1 μM (20), respectively. SEA reportedly shows ~10–30-fold higher affinity for MHC class II molecules than SEB on B cell lines (21). For MHC class II—transfected L cells, Kₐ's for SEA range from 82 to 143 nM (25). DR alleles have been shown to differ in their ability to bind SEA (26). Since our dendritic cells were obtained from buffy coat preparations, it was possible to correlate the Kₐ's measured to the DR phenotype. It is likely that a broad range of DR phenotypes was studied, as reflected by the range of Kₐ's obtained (Table 1). Substantial differences in on-rates for SEA at 4°C vs. 37°C have been described (21). Although large differences in Kₐ's at these two temperatures were not detected here, we did not directly measure the on-rate as a function of time.

When dendritic cells or Cox cells were treated with an anti—class II antibody (L243 or 9.3C9), before and during the SEA binding assay, at least 50% of the specific binding was inhibited (data not shown), similar to findings with TSST-1 on tonsillar B cells (19). Control anti-CD14 antibody had no effect on binding of SEA to Cox cells (data not shown).

Interestingly, the class II—negative Ket/BLS1 cell line obtained from a patient with bare lymphocyte syndrome (16) showed specific binding of 125I-SEA, but this only became detectable at a relatively high concentration, 15 nM, and saturation could not be achieved (Fig. 1, A and B). An accurate estimate of these apparently low affinity superantigen-binding sites on the Ket cell line could not be determined, even when the numbers were increased 10-fold. Thus, besides binding to MHC class II—dependent, saturable high affinity sites, SEA also uses an MHC class II—independent pathway to interact with T cells, as previously suggested for SEB (27).

Dendritic cells were then compared with other primary APCs from blood, i.e., B cells and monocytes. These APCs are known to express lower levels of MHC class II products than dendritic cells using cytofluorographic assays (6, 15, 28). When low numbers of cells were used (10⁶/assay), as is required for dendritic cells, which are a trace cell population in blood, saturable binding could only be achieved with dendritic and B cells (Fig. 1 C). The B cells bound an average of 64,000 SEA molecules (range of 2.3 × 10⁶ to 1.2 × 10⁷), i.e., 30 times less than dendritic cells. To compare dendritic cells with monocytes more effectively, it was necessary to increase the number of monocytes per binding assay to 2 × 10⁶. Monocytes bound only 4,500–14,000 molecules/cell (Table 1), much less than the level of binding to dendritic

| Table 1. Cellular Distribution of SEA Binding Sites |
|---------------------------------|-------------|----------|
| Cell type (no. of exps.)        | Mean no. of sites per cell (range) | Mean Kₐ ± SD |
| Cox (n = 5)                     | 3.2 × 10⁶ | 13.4 ± 9.3 |
| (n = 7)                         | (0.2 × 10⁶–6 × 10⁶)                 |
| Dendritic cells (n = 7)         | 1.9 × 10⁶ | 5.6 ± 4.5 |
| (n = 7)                         | (0.2 × 10⁶–3.6 × 10⁶)               |
| B cells (n = 4)                 | 6.4 × 10⁴ | 1.5 ± 0.4 |
| (n = 2)                         | (2.3 × 10⁴–1.2 × 10⁵)              |
| Monocytes (n = 2)              | 9.3 × 10³ | 1.7 ± 1.2 |
|                               | (4.5 × 10³–1.4 × 10⁴)              |

Kₐ values and number of SEA binding sites per cell were calculated as described in Fig. 1. For Cox cells and dendritic cells, 10⁶ cells were used per binding assay, while higher numbers (2 × 10⁶) were used for monocytes and B cells. The number of SEA binding sites on dendritic cells may be an underestimate, since the purity of our preparations ranged from 50 to 75% (Fig. 1).
cells. Scatchard plots calculated from several experiments demonstrated that the affinity of binding to monocytes was similar to that measured on dendritic cells and the Cox cell line (Table 1), indicating that the receptor on these populations (presumably MHC class II) is identical.

Quantitation of Dendritic Cell MHC Class II—Superantigen Complexes Necessary for T Cell Stimulation. The minimal number of class II molecules that must be occupied by antigen on a dendritic cell for T cell activation is not known. Studies with long-term APC and T cell lines suggest that the amount of MHC—peptide complexes could be very small (200–600/ APC), and the extent of MHC occupancy likewise ≤0.1% (1, 2). It has been speculated that the large numbers of class II molecules on dendritic cells (6, 15, 28) account for their potency in several primary responses (29). To determine how many class II molecules must be occupied by superantigen to stimulate primary T cells, we pulsed dendritic cells and other APCs with different doses of iodinated SEA. We then quantified the number of receptors bound at each dose, and added parallel aliquots of APCs in graded doses to purified blood T cells to evaluate their functional potential in a proliferation assay. Dendritic cells and Cox cells were equally stimulatory, whether pulsed with SEA or exposed continuously to the superantigen during coculture with T cells (Fig. 2 A). Ket cells were only effective in stimulating T cells when SEA was continuously present in the cultures, consistent with our data that these APCs have low affinity SEA binding sites (Fig. 1) and probably cannot capture SEA well in pulse protocols.

SEA-pulsed B cells and monocytes were much less efficient stimulators of T cell proliferation than dendritic cells, even at higher doses of SEA and substantially higher APC/T cell ratios (Fig. 2 B). Dendritic cells pulsed with 0.005 nM (5 pM) of SEA effectively stimulated T cells at ratios of 1:100–1,000, whereas monocytes or B cells pulsed with up to 50 nM SEA induced lower responses even at APC/T cell ratios of 1:30 (Fig. 2 B). Dendritic cells pulsed with 1,000-fold less SEA (0.050 vs. 50 nM) bind similar levels of SEA per cell, relative to B cells, but are effective at <1/30 the number of B cells required to generate an equivalent T cell response. For example, it was necessary to use 3.3 × 10³ B cells (APC/T, 1:30) pulsed with 50 nM SEA to attain a similar degree of T cell stimulation as that obtained with 100 dendritic cells (APC/T, 1:1,000) pulsed with only 50 pM of superantigen.

B cells and monocytes did stimulate T cells more effectively if SEA were continuously present in culture and at relatively high doses. Interestingly, monocytes appeared to be more efficient than B cells in this situation, even though the former bind significantly less SEA (Fig. 1 C) and have fewer MHC class II molecules on their surface (6, 15, 28). Our studies deal with primary B cells in blood, but it is possible that activated B cells would resemble B cell lines and stimulate superantigen responses better than resting blood B cells. However, is not yet evident that superantigen or antigen will stimulate a sufficient number of B lymphocytes to permit such a function for the activated B cell in vivo.

The dose-response curves that describe the binding of SEA to dendritic cells, and the efficacy of superantigen-pulsed APCs in stimulating T cells, are strikingly different (Fig. 3). At stimulator-to-responder ratios of 1 dendritic cell to 100 T cells, half-maximal proliferation was achieved after an SEA pulse of only 50 pM, whereas half-maximal occupancy of receptors occurred at 5 nM. Therefore one needs 100-fold less superantigen to induce half-maximal proliferation of T cells than to occupy half the available affinity receptors. At 50 pM SEA, the dose required for half-maximal stimulation, only 1% of receptors (presumably MHC class II) are bound with superantigen. At 5 pM, where strong T cell responses are still evident, only 0.1% of receptors or ~2,000 occupied MHC molecules are required (see Table 1).

Functional Features of SEA Binding to Dendritic Cells. Since dendritic cells were active APCs when pulsed with low levels of superantigen, a high affinity binding site, most likely MHC class II, seemed essential. Addition of anti-MHC class II mAbs to dendritic cell–T cell cultures in the presence of SEA virtually abrogated presenting ability to T cells (Fig. 4; note log axis, block is >95%). Similar results were obtained with the
Cox cell line, but not the MHC class II-negative Ket cell line (data not shown), where the induction of T cell proliferation was minimally affected by the addition of anti-MHC class II antibodies.

We next determined if dendritic cells could retain immunogenic MHC-superantigen complexes for long periods. SEA-pulsed dendritic cells, in fact, retained detectable 125I-SEA (not shown) and could activate T cells if tested 48 h after the pulse (Fig. 5).

Subsets of blood T cells can be distinguished on the basis of isoforms of the leukocyte common antigen CD45. The CD45RO⁺ CD45RA⁻ subset contains most antigen-primed or memory T cells, whereas CD45RO⁻ CD45RA⁺ cells do not have reactivity to these recall antigens and are considered to be naive (30, 31). Superantigens have been shown to stimulate both populations (32), but the role of dendritic cells has not been evaluated. We separated blood T cells into CD4⁺ naive and memory subsets and measured their responses to SEA presented by dendritic cells. Both the naive and memory T cells responded to dendritic cells pulsed with 5 pM to 0.5 nM SEA (Fig. 6). The naive population was more limited in its response at the lower doses of SEA, but was equivalent to the other populations at higher doses in several experiments. To monitor the cell populations, we verified that the memory cells responded to the recall antigen *C. albicans* while the naive cells were depleted of recall reactivity (Fig. 6, inset). These results confirm that quiescent or resting T cells respond significantly to a small number of superantigen–MHC class II complexes on dendritic cells.

**Figure 2.** Presentation of SEA by different APCs. (A) SEA-pulsed dendritic cells and Cox cells, but not MHC class II-negative Ket cells, efficiently induce T cell proliferation. Dendritic cells, Cox cells, and Ket cells were added to purified blood T cells in the continuous presence of 125I-SEA (nonpulsed APCs), or were pulsed for 1 h at 4°C with 0.005–50 nM of 125I-SEA, washed extensively, and added back to T cells (pulsed APCs). The APC/T ratio was 1:100. Proliferation was determined on day 3 with the addition of 1 μCi [3H]thymidine for 12 h to triplicate microwells (mean cpm). (B) Dendritic cells are more potent APCs than B cells or monocytes in stimulating T cell responses to SEA. Dendritic cells (DC), monocytes (Mφ), and B cells derived from the same donor were pulsed with SEA at 37°C, or the APCs were added directly to T cells in the continuous presence of SEA at various APC/T cell ratios (1:30–1,000).
Dendritic cells pulsed with low doses of SEA retain superantigen for at least 2 d in culture. After pulsing with 0.5 nM SEA, dendritic cells were left in culture for 0, 1, or 2 d and then added to purified T cells at various APC/T cell ratios. Proliferation was determined on day 3 after an overnight pulse with 1 µCi of [³H]TDR.

Figure 5. Dendritic cells pulsed with SEA retain superantigen for at least 2 d in culture. After pulsing with 0.5 nM SEA, dendritic cells were left in culture for 0, 1, or 2 d and then added to purified T cells at various APC/T cell ratios. Proliferation was determined on day 3 after an overnight pulse with 1 µCi of [³H]TDR.

Discussion

Dendritic cells represent a widely distributed system of APCs that is particularly active for the induction of T cell–mediated immunity (reviewed in reference 29). Among the many stimuli that are effectively delivered are several microbial superantigens (6). By isolating dendritic cells from human blood, and by using superantigens as the ligand to be presented to T cells, several quantitative features of APC function have become apparent and are discussed individually here. Essential to the analysis is the fact that superan-
tigens do not appear to undergo processing but instead directly engage MHC class II molecules on the APC and subsequently bind to Vβ segments of the TCR (4, 5, 21, 40). This enables one to pulse APCs with superantigen, and then relate amounts of APC-associated superantigen to subsequent lymphocyte stimulation. This method has provided quantitative information on signal one for the first time using quiescent T cells and APCs from humans. In addition, we have been able to compare superantigen binding and presentation by APCs quantitatively and to evaluate the contribution of costimulator molecule pairs (signal two) when known amounts of ligand are bound to the APC.

Pulse, rather than continuous, exposure to superantigen is critical for the analysis presented here, since it permits direct measurement of the maximal amount of superantigen that is conveyed by APCs during T cell stimulation. Mollick et al. (25) estimated that small amounts of bound SEA were sufficient for T cell stimulation by L cells transfected with MHC class II molecules. However, the SEA was applied continuously rather than in a pulse. During continuous exposure, APCs could accumulate significant amounts of SEA after binding, and reservoirs of low affinity binding sites could contribute additional SEA.

Dendritic cells bind much larger amounts of superantigen than do other APCs like monocytes and B cells (Fig. 1 C and Table 1). This might have been predicted from the higher
levels of MHC class II molecules on dendritic cells (6, 15, 28), assuming the superantigen binding sites are unoccupied. Because dendritic cells comprise ~0.1% of blood leukocytes but bind >30 times more superantigen than B cells and >200 times more than monocytes, it is necessary to isolate this trace subset of APCs to appreciate the efficacy with which superantigens can stimulate the growth of primary T cells. Not only are dendritic cells much more efficient on a per cell basis than other primary APCs in stimulating T cells to proliferate to superantigen (Fig. 2 B), they also use fewer MHC class II molecules. We estimate that 25–30-fold fewer class II molecules are engaged by dendritic cells compared with B cells to attain a similar degree of T cell stimulation (Fig. 2 B).

The picomolar concentrations of superantigen that are required to pulse dendritic cells are 10⁶-fold less than the micromolar levels required for conventional soluble antigens (8, 41). Since superantigens bind directly to MHC class II products (20–22), while most conventional antigens probably do not bind directly and must first be processed, it is possible that 1/10⁶ of a conventional antigen load is successfully processed by dendritic cells to form MHC class II–peptide complexes.

High levels of MHC class II, a uniform feature of dendritic cells from many different tissues and species (29), have implied that high levels of signal one underlie their efficacy as APCs. However, this proved not to be the case for superantigens at least in vitro. An average of just 2,000 bound molecules of SEA per dendritic cell induced strong responses in quiescent T cells (Fig. 3 A). When one considers that 1 dendritic cell can stimulate 10 or more T cells simultaneously within cell clusters (42), and that our estimates of bound superantigen could be high if SEA were shed, metabolized, or bound in part to non-MHC products, then the number of MHC-associated SEA molecules that are required to stimulate a primary lymphocyte is remarkably small, possibly <200 molecules per T cell. This is true for both naive and memory subset of APCs to appreciate the efficacy with which superantigens have been described (37). However, in these studies, either high doses of SEA, added on a continuous basis, or APCs (B cell line) were used. Under these conditions, the amount of SEA that binds directly to APCs cannot be quantified. It is possible that higher amounts of ligand may be less dependent on accessory molecules, as postulated for the CD2–CD58 (43) and CD11a–CD54 interactions (44).

These data provide insight on the exceptional qualities of the dendritic cell in eliciting primary responses. First, dendritic cells can present low amounts of the MHC class II–SEA complex (signal one) and several costimulatory molecules or signal two (ICAM-1, LFA-3, B7/BB1 [11]) simultaneously to T cells to which dendritic cells bind efficiently and for days (13). Second, the high number of class II molecules (~2 x 10⁶) on its surface would permit the dendritic cell to bind several hundred peptides simultaneously, at concentrations that would be functionally active. For example, 1,000 peptides, each occupying 2,000 class II molecules, could be accommodated. Thus, even if a diversity of peptides are generated from complex antigens, assuming that they can be bound by class II molecules, many could be presented by the dendritic cell at one time.

If large amounts of signal one are not required for dendritic cells to initiate a primary T cell response, what might be the function of large amounts of MHC class II on dendritic cells? One function would be to allow dendritic cells to capture ligand (superantigens and peptides) at very low external concentrations. A second is that high levels of superantigen might lead to better retention of antigen in the long term especially in situ. Superantigens and possibly other ligands dissociate from MHC molecules at a finite rate (22). Dendritic cells pulsed with 0.5 nM SEA are stimulatory for T cells that are added as much as 2 d later (Fig. 5). These possibilities are relevant in vivo, where dendritic cells capture antigens more efficiently than other APCs (45) and can migrate to the T cell areas of lymphoid tissues (46) and prime MHC-restricted T cells, a response that proceeds over a 5–7-d period (8). Injected dendritic cells that carry the endogenous Ms superantigen can tolerize developing thymocytes to Ms antigens over at least 1 wk in situ (47, 48). Third, high levels of MHC products on dendritic cells would also enable these APCs to display many different peptides or superantigens, which may be relevant in responses to complex microbial and cellular stimuli. Dendritic cells are found in relatively large numbers in synovial effusions (49). If superantigens are important in rheumatoid arthritis as suggested (50, 51), the ability of dendritic cells to carry small amounts of superantigen in a form that is highly stimulatory for T cells suggests that they would be important APCs for inducing immune responses to these molecules in vivo.
We thank Dr. K. A. Smith for considerable advice and discussions on the binding studies, Drs. J. S. Lee, P. Cameron, and P. C. L. Beverley for the Ket/BSL1, Cox and the UCHL1 cell lines, respectively, J. Adams for graphics, and E. Feller for technical assistance.

This work was supported by grants from the National Institutes of Health (AR-39552 [N. Bhardwaj], AI-24775 [R. M. Steinman], CA-00961 and AI-26875 [J. W. Young]); the Irma T. Hirschl Trust (N. Bhardwaj); the Francis Florio Fund, Blood Diseases Research Program, New York Community Trust (J. Young); and the Danish Medical Research Council (J. Bagers).

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Received for publication 25 February 1993 and in revised form 4 May 1993.

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