Dendritic Cells Are Potent Antigen-presenting Cells for Microbial Superantigens

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Summary

Dendritic cells are a small subset of human blood mononuclear cells that are potent stimulators of several T cell functions. Here we show they are 10–50-fold more potent than monocytes or B cells in inducing T cell responses to a panel of superantigens. Furthermore, dendritic cells can present femtomolar concentrations of superantigen to T cells even at numbers where other antigen-presenting cells (APCs) are inactive. Although dendritic cells express very high levels of the major histocompatibility complex products that are required to present superantigens, it is only necessary to pulse these APCs for 1 hour with picomolar levels of one superantigen, staphylococcal enterotoxin B, to maximally activate T cells. Our results suggest that very small amounts of superantigen will be immunogenic in vivo if presented on dendritic cells.

Microbial superantigens are of major clinical interest because they cause a spectrum of disease in both humans and animals. For example, in humans, proteins derived from *Staphylococcus aureus* induce gastroenteritis, toxic shock syndrome, and the scalded skin syndrome (1, 2). In rodents, the only species of *Mycoplasma* known to produce a superantigen, *Mycoplasma arthritidis*, causes a chronic inflammatory polyarthritis (3, 4).

Superantigens share several important features that distinguish them from conventional antigens. Superantigens are potent mitogens for both human and mouse T cells because they typically stimulate large numbers of T cells bearing particular TCR Vβ gene products (5, 6). T cell responses to superantigens are not MHC restricted in the classical sense since the superantigen is bound and presented by a variety of allelic and isotypic MHC molecules (7). Superantigens appear to bind to sites on MHC class II molecules that are located outside the peptide binding groove (8, 9). Where it has been studied, binding to MHC class II antigens is half-maximal at nanomolar to micromolar levels of superantigen (10–12).

Despite the considerable information available regarding binding of superantigens to MHC molecules, there is little known comparing the presenting function of different types of MHC class II$^+$ cells, particularly dendritic cells. We have compared the capacity of blood dendritic cells with B cells and monocytes to present staphylococcal toxins and *M. arthritidis* mitogen (MAM)[1] to human T cells. We find that dendritic cells are 10–50-fold more efficient than other APCs and require only trace levels of superantigen to initiate T cell responses.

Materials and Methods

Culture Medium. RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 5% human serum, and 50 μg/ml gentamycin was used.

Mitogens. Staphylococcal enterotoxins (SEs) A, B, and E, and toxic shock syndrome toxin 1 (TSST-1) were purchased from Toxin Technology (Madison, WI). The mitogens were aliquoted to 10 μg/ml in RPMI and stored at −20° until use. The mitogen MAM, a culture supernatant of *M. arthritidis*, was used at a dilution of 1:4,000.

Preparation of Lymphoid Cells. Lymphoid cells were isolated from buffy coats obtained from the New York Blood Center and separated into T cell–enriched (erythrocyte rosette-positive [ER$^+$]) and T cell–depleted (ER$^-$) fractions by rosetting with neuraminidase-treated SRBC.

T Cells. The ER$^+$ cells were then depleted of non-T cells by panning on human Ig-coated plastic dishes (13) and by panning

[1] *Abbreviations used in this paper:* ER, erythrocyte rosette; MAM, *Mycoplasma arthritidis* mitogen; SE, Staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1.

Ia$^+$ cells with the mAb 9.3C9 (no. HB180; American Type Culture Collection, Rockville, MD). The resulting T cell preparations failed to proliferate to optimal doses of superantigens indicating negligible contamination with APCs.

Monocytes. The ER$^-$ fraction was adhered to plastic dishes for 60–90 min and nonadherent cells were removed with four to five washes. Firmly adherent monocytes were collected by incubating the dishes with cold PBS for 15–20 min and gentle aspiration. Alternatively, the adherent cells were cultured with fresh medium overnight, at which time the cells were easily dislodged from the plastic surfaces.

B Cells and Dendritic Cells. Nonadherent ER$^-$ cells were depleted of residual monocytes by panning on Ig-coated plates and then layered onto 14.5% metrizamide (13). After sedimentation at 650 *g* for 10 min at room temperature, B cells and a few NK cells "pellet" as the high density fraction, while dendritic cells float as the low density fraction (13). The cell fractions were washed in successively less hypertonic washes before being tested.

Cell Sorting. In some experiments, the APC populations were purified further by cell sorting on a FACStar Plus® (Becton Dickinson & Co., Mountain View, CA). 10^6 cells were stained with a cocktail of PE-conjugated mAb (10 μl of stock-purchased mAb in 1 ml of medium) on ice for 45 min, washed three times, and sorted (13). B cells were collected as small Leu-4-, Leu-11-, Leu-19-, Leu-M3-negative cells; dendritic cells were large Leu-4-, Leu-11-, Leu-12-, Leu-16-, Leu-19-, Leu-M3-negative cells; and monocytes were large Leu-4-, Leu-11-, Leu-12-, Leu-16-, Leu-19-negative cells. The sorted cells were analyzed for purity by staining with PE-conjugated mAb to B cells (Leu-12 and -16), monocytes (Leu-M3), or with a cocktail (Leu-4-CD3, Leu-12-CD19, Leu-16-CD20, Leu-19-CD16, Leu-M3-CD14; Becton Dickinson & Co.). This verified that our populations were >95% pure.

Lymphocyte Proliferation Assays. Responses to microbial antigens were induced by adding graded doses of viable irradiated (3,000 rad 137 Cs) APCs to 1.5 × 10^5 syngeneic T cells in 96-well flat-bottomed plates (Costar, Cambridge, MA) in 0.2 ml of medium. In some experiments, APCs were not irradiated. Proliferation was measured by the addition of 4 μCi/ml [^{3}H]thymidine (6.0 Ci/mM) for 16 h on day 2 or 3 of culture. For primary MLRs, allogeneic T cells were used. [^{3}H]Thymidine was added from 120–136 h. Results are expressed in counts per minute as an average of triplicates. For pulsing studies, either T cells or APC were incubated with microbial antigens for 1 h at 37°C, washed three times, and added to microtiter wells as above. The cells were pulsed at 1–2 × 10^6/ml in sterile 1.5-ml microcentrifuge polypropylene tubes with doses of SEB that ranged from 10 pg/ml to 1 μg/ml.

Results

Dendritic Cells Are Potent APCs for a Panel of Microbial Superantigens. Blood monocytes, B cells, and dendritic cells were compared for their ability to present the staphylococcal proteins SEA, SEE, SEB, TSST-1, and the mitogen MAM. When present at 1 cell per 30–300 T cells, dendritic cells were far more efficient at stimulating T cell proliferation than monocytes or B cells (Fig. 1; representative of five experiments). At doses of 5–10 ng/ml, maximal T cell proliferation to the staphylococcal toxins was observed at days 2–3 of culture. The T cell proliferative response to MAM was considerably slower, reaching maximal levels at days 4–5. For this superantigen, monocytes and B cells were strikingly less efficient than dendritic cells (Fig. 1 *B*).

In the presence of superantigens, dendritic cells appeared to engage T cells in clusters or aggregates as described previously for other T cell responses; e.g., the MLR (14). The superiority of dendritic cells to other APCs could not be accounted for by time course differences since pulsing cultures at later intervals showed similar differences between these cells (data not shown). In the allogeneic MLR, dendritic cells are the most potent APC when compared with other cells (14). A corresponding allogeneic MLR (Fig. 1 *A*) with the same populations of APCs is shown for comparison.

Trace Numbers of Purified Dendritic Cells Induce T Cell Mitogenesis to Superantigens. We sorted our dendritic cell populations to be sure that contaminating cells, e.g., B cell blasts,

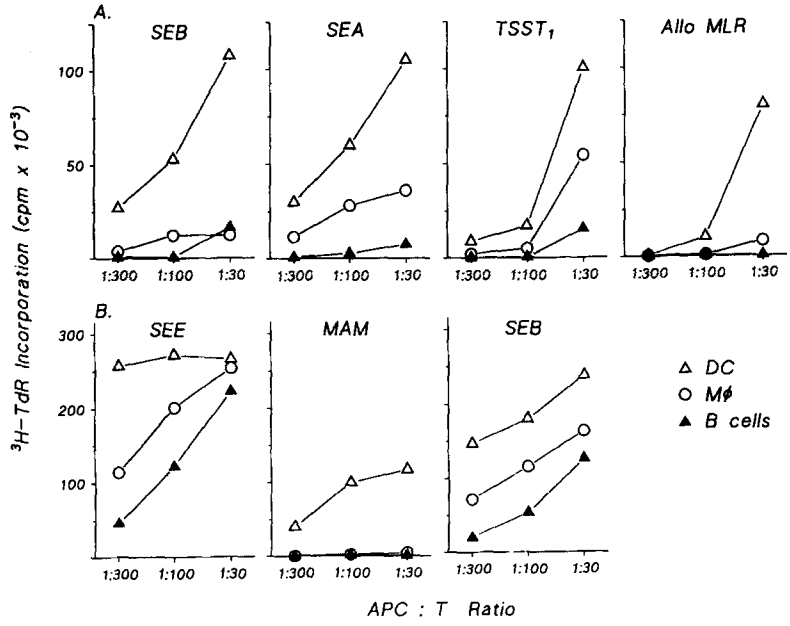

Figure 1. Presentation of superantigens to T cells by enriched populations of APCs. Graded doses of APCs (dendritic cells, B cells, monocytes) were added to T cells (1.5 × 10^5) in the presence of SEB or SEA (5 ng/ml), TSST-1 (10 ng/ml), and MAM (1:4,000). T cell proliferation is measured as [^{3}H]TdR incorporation in cpm (average of triplicates). Activity of the APC populations in an allo-MLR is shown for comparison.

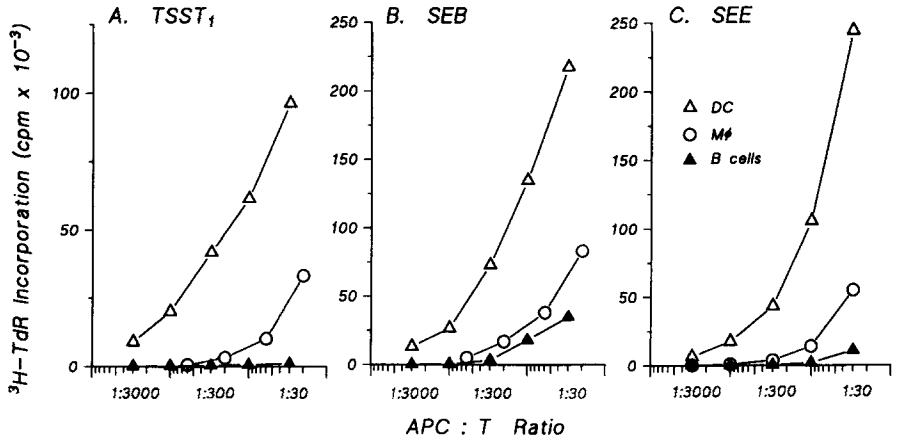

Figure 2. Purified dendritic cells are the most potent APCs for superantigens. APCs were purified by sorting on a FACStar® as described (Materials and Methods) and added at graded doses to T cells with a panel of superantigens.

were not responsible for the efficient superantigen presentation. When compared with sorted B cells and monocyte preparations, dendritic cells were consistently 10–50-fold more potent in presenting TSST-1 (10 ng/ml) and SEB (5 ng/ml; Fig. 2, *A* and *B*). Similar observations were made with SEE (0.5 ng/ml; Fig. 2 *C*). An important finding was that sorted dendritic cells were effective even when present at 1 cell per 3,000 T cells. At these doses, dendritic cells did not stimulate allogeneic T cells in the MLR (data not shown). Thus, dendritic cells can present superantigens at concentrations where other T cell–dependent responses are not apparent in vitro. These experiments also consistently showed that B cells were less potent than monocytes in presenting superantigens. With TSST-1, especially, B cells were poorly stimulatory.

Femtomolar Concentrations of Staphylococcal Enterotoxins Can Be Efficiently Presented by Dendritic Cells. The ability of different APCs to stimulate T cells in the presence of a wide range of enterotoxin concentrations was evaluated (Table 1). At picomolar to femtomolar concentrations of SEE, dendritic cells induced T cell proliferation even when present in limiting numbers (1:300). In fact, the data suggest that dendritic cells would be effective at doses of SEE <5 pg/ml (170 fM). Monocytes and B cells stimulated T cells at femtomolar concentrations of SEE, but were less efficient, and relatively high stimulator to responder ratios (1:10–30) were required.

Expression of MHC Class II Antigens by Human Blood APCs. Superantigens bind to MHC class II molecules on APCs (11, 12, 15, 16). It was therefore of interest to compare

Table 1. *Comparison of Different APCs with Low Concentrations of Superantigen*

APC	APC/T cell ratio	T cell proliferation in presence of SEE			
		5 ng/ml	500 pg/ml	50 pg/ml	5 pg/ml
		cpm × 10^{-3}			
Monocytes	1:10	359,367	274,793	160,196	67,733
	1:30	183,170	126,238	44,294	14,293
	1:100	68,508	35,527	11,566	4,085
	1:300	23,997	13,843	2,926	601
B cells	1:10	224,984	148,001	71,166	31,315
	1:30	116,143	60,328	32,574	10,527
	1:100	33,861	14,259	6,183	1,351
	1:300	10,333	1,929	536	275
DC	1:10	379,401	323,158	330,666	274,365
	1:30	357,553	308,461	260,571	190,876
	1:100	274,187	212,873	163,697	105,577
	1:300	108,316	91,978	66,717	36,971

5ng/ml (170 pM) to 5 pg/ml (170 fM) concentrations of SEE were added to graded doses of APC and T cells (1.5×10^5). T cell proliferation is measured as [^{3}H]TdR incorporation in cpm (average of triplicates).

the relative expression of class II antigens on different human APCs (monocytes, B cells, dendritic cells). A representative experiment of three similar results is shown in Fig. 3. Monocytes, obtained from ER^- cells by plastic adherence, were >90% pure as judged by staining with mAb to CD14. B cells and dendritic cells were derived from ER^-, FcR^- cells after layering on metrizamide gradients. The high-density fraction was primarily $CD19/20^+$ B cells. The low-density fraction was 30–80% dendritic cells; i.e., large irregularly shaped cells that stained brightly with mAbs to class II MHC products (HLA-DR, -DQ, and -DP) but not with a cocktail of mAb to monocytes (CD14), NK cells (CD16, CD56), B cells (CD19, CD20), or T cells (CD3). Compared with the other APCs studied here, dendritic cells expressed 10–100-fold more DR, DP, or DQ molecules, as detected by intensity of staining (Fig. 3, *bottom three panels*).

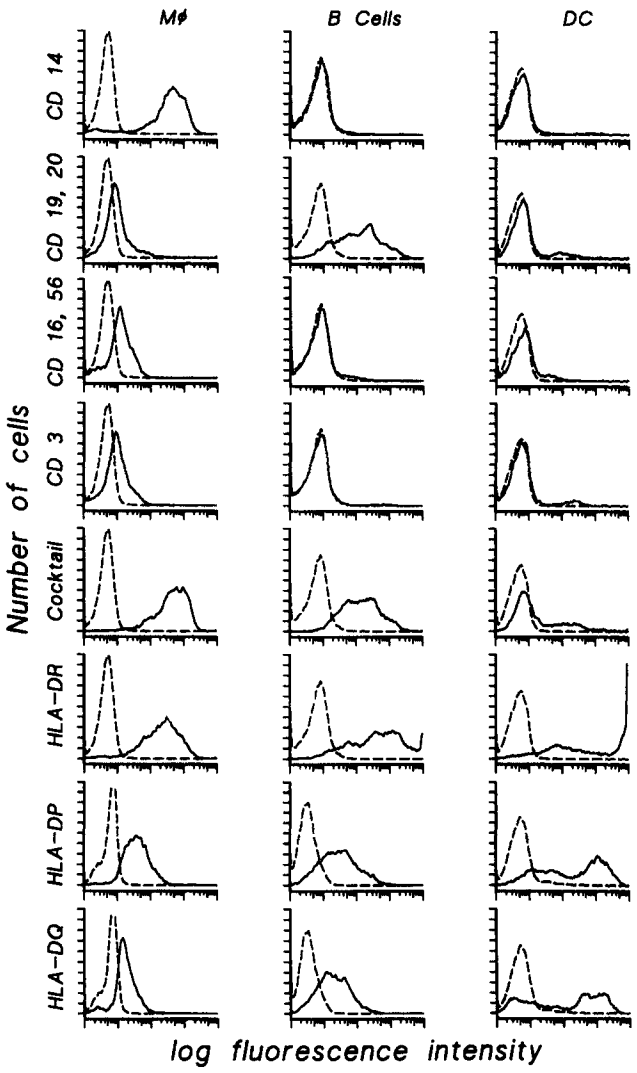

Figure 3. Ia antigen expression on enriched monocytes, B cells (pellet), and dendritic cells. APC populations were stained with a panel of mAbs (see Results) and analyzed for purity and Ia antigen expression. A representative experiment is shown. Monocytes ($CD14^+$) were >90% pure. The pellet fraction consisted of mostly B cells ($CD19/20^+$, 80%), while dendritic cells were >70% pure. Note that the expression of DR antigens for dendritic cells is off scale (third column, sixth row).

Superantigen Binding Activity of Dendritic Cells. The above results suggest that dendritic cells are more efficient at presenting superantigens because of their higher expression of Ia antigens. To gain a sense of the degree of occupancy of class II, we did pulsing experiments with graded doses of SEB. Dendritic cells and B cells were pulsed with femtomolar to nanomolar concentrations of SEB for 1 h at 37°C, washed extensively, and added in graded doses to purified T cells. An additional group of APCs (nonpulsed) were directly added in the continuous presence of T cells and SEB. At stimulator/responder ratios of 1:100, maximal T cell stimulation was achieved at 10–100 ng/ml for nonpulsed and pulsed cells (Table 2). Half-maximal stimulation was seen when dendritic cells were pulsed at 1 ng/ml (pM) of SEB. Dendritic cells pulsed with even femtomolar (10 pg/ml) concentrations of superantigen stimulated T cells to proliferate. Published data show that the K_d for SEB is 10^{-6} (11). Therefore, our results suggest that only small amounts of superantigen are needed on dendritic cells to maximally stimulate T cells. In contrast to dendritic cells, SEB-pulsed B cells failed to stimulate T cells even at stimulator/responder ratios of 1:30 (data not shown).

Discussion

Antigen presentation by MHC products on dendritic cells has been studied in the allogeneic MLR (14), and in responses to nominal antigen (17) or self-MHC (18). In all of these systems, dendritic cells are effective at very low numbers, e.g., at stimulator/responder ratios of 1:100. Other APCs are considerably less effective or are required at considerably higher numbers. Here we show that dendritic cells are also potent APCs for several superantigens. They are 10–50-fold more effective than monocytes or B cells, and very low numbers of dendritic cells (stimulator/responder ratios of 1:3,000) are effective. At these trace numbers, dendritic cells are not stimulatory in the allogeneic MLR. Our data confirm that femtomolar concentrations of superantigen are sufficient to stimulate T cells (15, 19). In general, previous studies evaluating superantigen presentation by APCs have used B cell lines, B cells, monocytes, or Ia-transfected fibroblasts (5, 16, 19–22). In most cases, however, it has been necessary to use these APCs at high stimulator/responder ratios (generally from 1:100 to 1:2.5) to elicit responses from T cells.

Dendritic cells were consistently better than monocytes or B cells in presenting staphylococcal toxins and MAM. With MAM, however, we noted even greater differences in the efficiency of presentation relative to monocytes and B cells (Fig. 2 *B*). It is possible that MAM is presented differently by dendritic cells than other superantigens. Alternatively, MAM may bind with variable efficiency to different class II MHC isotypes (e.g., DQ or DP) that are preferentially expressed on dendritic cells (Fig. 3).

The efficiency with which dendritic cells present superantigen to T cells is consistent with the degree of Ia antigen

Table 2. *Dendritic Cells Pulsed with Femtomolar Concentrations of SEB Induce T Cell Proliferation*

Exp.	APC	APC/T cell ratio	T cell proliferation in response to SEB					
			1 μg/ml	100 ng/ml	10 ng/ml	1 ng/ml	100 pg/ml	10 pg/ml
			cpm × 10^{-3}					
1	DC,	1:100	72,024	105,297	106,942	92,923	84,799	69,903
	nonpulsed	1:300	59,137	54,829	56,426	45,937	54,288	44,430
		1:1,000	25,552	18,504	26,502	21,589	22,746	19,987
	DC,							
	pulsed	1:100	141,994	147,489	110,475	52,824	26,375	30,095
		1:300	74,837	67,289	44,411	18,660	8,196	4,073
		1:1,000	44,971	29,179	10,446	3,474	717	505
2	DC,							
	nonpulsed	1:100	98,935	95,496	79,312	61,043	59,168	
		1:300	54,743	41,666	37,746	28,403	25,769	
		1:1,000	23,389	20,690	14,604	7,372	8,945	
	DC,							
	pulsed	1:100	107,619	83,579	66,737	61,694	38,776	
		1:300	38,506	33,191	24,811	23,073	8,792	
		1:1,000	16,347	13,981	9,546	7,456	2,001	

Dendritic cells were pulsed with varying doses of SEB, 1 μg/ml (35 nM) to 10 pg/ml (350 fM), for 1 h at 37°C, washed, and then added in graded doses to T cells. Nonpulsed dendritic cells were added directly to T cells after which SEB was aliquoted in the same concentrations as above. T cell proliferation is measured as [^{3}H]TdR incorporation in cpm (average of triplicates). For cultures containing nonpulsed dendritic cells, background proliferation counts of T cells alone plus SEB have been subtracted from each value. [^{3}H]TdR incorporation of pulsed dendritic cells cultured in the absence of T cells was 44–1,612 cpm.

expression that is expressed on their surface. Dendritic cells express from 10- to 100-fold more DR, DQ, DP on their surfaces than B cells or monocytes. Thus, dendritic cells would have a major advantage in binding or presenting superantigens to T cells since they: (*a*) would quantitatively bind more superantigen, and (*b*) express all three class II isotypes at high densities.

However, it appears that only a few of the Ia molecules on dendritic cells are utilized for presentation of superantigen. Dendritic cells pulsed for only 1 h with 10–100 ng/ml of SEB are as efficient as those exposed to the same concentrations of SEB continuously in culture for 3 d (Table 2). We find that pulsing dendritic cells with these picomolar concentrations of SEB leads to rapid and extensive clustering with T cells. Half-maximal proliferation of T cells is achieved after a pulsing dose of 1 ng/ml or 35 pM, whereas the published K_d for SEB, as determined on B cell lines, is 1 μM (11). In fact, pulsing dendritic cells with 10 pg/ml (350 fM) still results in T cell activation (Table 2). Furthermore, since dendritic cells pulsed with picomolar concentrations of SEB can activate T cells when present at 1 cell per 1,000 T cells, only trace numbers are necessary. While other investigators have shown that superantigen-pulsed APCs stimulate T cells (23–25), in general, considerably higher doses have been used (1 μg/ml). In one other study, Ia$^+$ B cell lines were shown to present SEA to T cells after a 1-h pulse with 1 ng/ml, but graded doses of superantigen or APCs were not tested (15). Our data suggest that for dendritic cells, a very small fraction of MHC class II molecules need to be occupied to stimulate a T cell. In some respects, superantigen presentation resembles peptide presentation where small amounts of antigen suffice and can saturate the system. Thus, superantigen stimulation of T cells may be a useful and simple model to study the accessory functions that allow dendritic cells to stimulate so well.

We have begun to assess the contribution of other molecules that are known to be involved in APC–T cell interactions. Blood dendritic cells express high levels of two β-2 integrins (LFA-1/CD11a and CD11c) and the adhesins leukocyte function-associated antigen 3 (LFA-3) and intercellular adhesion molecule 1 (ICAM-1) (13). Interaction of superantigens with Ia antigens on B cell lines leads to activation of LFA-1 molecules and more efficient homotypic aggregation of the cells (26). ICAM-1 has recently been shown to provide co-stimulator function for SEA-specific MHC class II–dependent activation of resting CD4$^+$ T cells (27). Furthermore, dendritic cells express B7/BB1, the receptor for CD28, and these antigens are important participants in T cell acti-

vation and the generation of the allogeneic MLR by dendritic cells (Young, J. W., K. Koulova, S. A. Soergel, E. A. Clark, R. M. Steinman, and B. Dupont, manuscript submitted for publication). Thus, it is possible that the abundant expression of Ia antigens on dendritic cells is not the only feature that permits them to bind and present superantigens at low concentrations or trace numbers in vitro. Consistent with this interpretation, we and others (5, 19) have shown that B cells, which express a higher degree of Ia antigens on their surfaces than do monocytes (Fig. 3), appear to be less efficient than monocytes in superantigen presentation. Furthermore, when B cells are pulsed with as much as 1 μg/ml of SEB, they are nonstimulatory even at stimulator/responder ratios of 1:30 (data not shown). Superantigens have profound effects when given in vivo. Neonatal mice given SEB delete Vβ3$^+$ and Vβ8$^+$ T cells (28). Injection of SEB into mice results in expansion followed by clonal anergy and a partial deletion of Vβ8.1$^+$ CD4$^+$ T cells (29). Patients suffering from toxic shock syndrome have elevated levels of Vβ2$^+$ T cells (30). Massive stimulation of these T cells in vivo leads to the release of mediators such as TNF and IL-2, which may be responsible for inducing shock-like syndromes after infection (31). Likewise, APCs are stimulated to secrete IL-1 and TNF after stimulation with superantigens, and this compounds the clinical disease (32, 33). Because of the need for low levels of superantigen, our findings raise the possibility that dendritic cells are important APCs during infection by organisms that generate superantigens. Dendritic cells can stimulate T cells in a Vβ-selective manner (data not shown), and they and their counterparts have been identified in blood, gut, skin, heart, and joint effusions (34–38). These are all organ systems involved in diseases that are likely to be caused by microbial superantigens. Furthermore, dendritic cells are known to migrate from tissues to lymph and blood, and then lymph nodes and spleen where presentation to T cells would occur (39). For limiting or subsaturating concentrations of superantigen, dendritic cells are likely to be the first avenue whereby these proteins are encountered in the body. In recent studies in mice, dendritic cells have been shown to present the endogenous superantigens (Mls) in vivo (40). Given these findings, it is possible that very low levels of superantigens can stimulate T cells and cause inflammation and tissue damage in situ.

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