A Quantitative Analysis of Antigen-presenting Cell Function: Activated B Cells Stimulate Naive CD4 T Cells but Are Inferior to Dendritic Cells in Providing Costimulation

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Summary

Ligation of CD28 on CD4 Th1 clones and freshly isolated mixtures of naive and memory CD4 T cells triggered their T cell receptors (TCR) is sufficient to induce the costimulatory signals necessary for interleukin 2 (IL-2) production by these cells. CTLA-4–reactive ligands expressed on antigen-presenting cells (APC) are critical in providing costimulatory signals to these T cell populations. We demonstrate that these activation characteristics apply equally to purified naive CD4 T cells. Because B cell blasts express CTLA-4–reactive ligands and high levels of adhesion and major histocompatibility complex class II molecules, they would be expected to engage both the TCR and CD28 and consequently stimulate IL-2 production by naive CD4 T cells. Using purified populations of cells in limiting dilution cultures, we have carried out a quantitative analysis of the interaction between naive CD4 T cells and either activated B or dendritic cells. We demonstrate that B cell blasts stimulate a high frequency of naive CD4 T cells. Slight differences in TCR signaling efficiency between the two APC types were observed. Even at optimal peptide concentrations, however, the amount of IL-2 made by individual T cells was fourfold lower in response to B cell blasts than to dendritic cells. This relative deficiency of activated B cells was due to their inability to optimally costimulate naive CD4 T cells.

The principal aim of this work was to examine the ability of various APC types to stimulate IL-2 secretion by naive CD4 T cells, with particular focus on activated B cells. Activated B cells can clearly stimulate many T cell hybridomas and clones, and freshly isolated populations of CD4 T cells from unprimed animals, which typically include both naive and memory T cells (1–3). In contrast, the question of whether activated B cells stimulate naive CD4 T cells remains controversial. Antigen-specific B cells are remarkably efficient at concentrating and processing antigen (4). Moreover, the specificity of B cells can influence the array of peptides generated during antigen processing (5–7). These characteristics have been suggested to allow B cells to increase the repertoire of T cell specificities observed during an immune response, a phenomenon called diversification or determinant spreading (8, 9). A potential consequence of this is that as B cells become activated during an immune response, B cells specific for an epitope shared between foreign and self antigen could process the self protein and present novel T cell determinants that have not been involved in intrathymic negative selection. Were activated B cells able to stimulate naive T cells, this would pose the risk of autoimmunity (10). To counter this, it has been proposed that primed B cells tolerize naive T cells. Were activated B cells tolerogenic, this could render peptide–carrier conjugates ineffective as T cell vaccines for individuals who already have carrier-primed B cells (11). Thus, the issue of whether activated B cells are stimulatory or tolerogenic for naive T cells is of both theoretical and practical concern.

Previous studies (12–17) have demonstrated a role for B cells in primary T cell responses in vivo. It is not clear in most of these studies, however, whether B cells directly present antigen to T cells or whether their involvement depends only on their production of antibody. Activated B cells have also been reported to stimulate naive T cells in vitro (18, 19). Because of the questionable reliability of the CD45RB isoform as a marker for naive T cells (20) and the large numbers of T cells and APCs used in this studies, it is difficult to be certain that the observed responses were due to interactions between naive T cells and activated B cells and not to contaminating memory T cells or non-B APCs. In contrast to these studies, there are two recent reports that activated B cells fail to prime (21) and instead tolerize naive T cells (22). The T cell populations directly examined in these studies, however, did not include peripheral CD4 T cells and may reflect distinct activation requirements of other T cell subsets. Thus, the issue of activated B cells as APCs for naive CD4 T cells remains unresolved and prompted the current study.

Materials and Methods

Animals. B10.A and CBA/J mice were purchased from Charles River Laboratories (Wilmington, MA) through a contract with the National Cancer Institute, National Institutes of Health (NCI NIH, Frederick, MD) and used between 2 and 6 mo of age. H-2$^{a/b}$, pigeon cytochrome c (pcc)[1]-specific and TCR-α/β, transgenic mice were derived by mating a (C57BL/6 × SJL)F$_1$ transgenic mouse (provided by Dr. S. Hedrick, University of California at San Diego, La Jolla, CA) to B10.A mice, then backcrossing transgenic mice with normal B10.A or C57BL/10 animals. Mice between the fifth and ninth backcross to the C57BL/10 background were used at 8–12 wk of age. These animals were housed in a barrier colony after embryo transfer at the fifth backcross generation. For priming, normal B10.A mice were injected subcutaneously with 10 nmoles of the COOH-terminal fragment 81-104 of pcc (Sigma Chemical Co., St. Louis, MO) emulsified in CFA (Difco Laboratories, Detroit, MI).

Culture Media and Reagents. Lymphoid suspensions were made in a standard balanced salt solution containing 5% FCS (Biofluids, Inc., Rockville, MD) and 100 μg/ml DNase (Sigma Chemical Co.). Cell culture was carried out at 37°C, 5% CO_2 in Eagle's Hanks' amino acid medium supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin, 100 U/ml streptomycin, 25 μg/ml gentamicin (BioWhittaker Inc., Walkersville, MD), 50 μM 2-ME (Sigma Chemical Co.), and an additional 1 mM sodium pyruvate, and 1X nonessential amino acids (Biofluids, Inc.). Mouse rIL-2 and GM-CSF were purchased from Pharmingen (San Diego, CA). Mouse rIL-4 was kindly provided by Dr. W. Paul (NIH, Bethesda, MD). Purified J11d.2 (α heat stable antigen [HSA]) (Pharmingen) and ammonium sulfate (Mallinckrodt Specialty Chemicals Co., Paris, KY)–concentrated supernatants of the following mAb-producing hybridomas were used for cell depletions: 3-1.5.5 (αCD8), J1j.10 (αThy-1.2), 10-2.16 (αI-A^k), Y-17 (αI-E^k), 33D1 (antispleen dendritic cells) (American Type Culture Collection, Rockville, MD), LICR.LAU.-RL172.4 (αCD4), and SW3A4 (αNK1.1, kindly provided by Dr. V. Kumar, University of Texas Southwestern Medical Center, Dallas, TX). Expression of class II and adhesion molecules was determined by staining with FITC-conjugated mAb to I-E, intercellular adhesion molecule 1 (ICAM-1), and LFA-1 (Pharmingen). CTLA4Ig refers to a fusion protein consisting of the extracellular portion of either mouse or human CTLA-4 spliced onto the CH2 and CH3 domains of human IgG1. mCTLA4Ig and hCTLA4Ig, generously provided by Dr. P. Linsley (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA), were used in this study with identical results. 37.51 hybridoma cells secreting αCD28 mAb were the kind gift of Dr. J. Allison (University of California, Berkeley, CA). GL1 (αB7-2) mAb was generously provided by K. Hathcock and Dr. R. Hodes (NIH).

Isolation of CD4 Lymphocytes. Nylon wool (Robbins Scientific Corp., Sunnyvale, CA) nonadherent lymphoid suspensions were incubated with 3-1.5.5, 10-2.16, Y-17, J11d.2, SW3A4, DNase, and Low-Tox-M rabbit C' (Cedarlane Laboratories Limited, Hornby, Ontario, Canada) and centrifuged over Lymphocyte-M (Cedarlane). Viable cells were stained with allophycocyanin-GK1.5 (αCD4), PE-IM7.8.1 (αCD44), and FITC-RR4-7 (αV$_\beta$6) or RR8-1 (αV$_\alpha$11) (Pharmingen) and sorted on the FACStar PLUS® (Becton Dickinson & Co., Mountain View, CA). Sorted cells were in some experiments dispensed at one cell per well using a FACStar® equipped with a FACS® Automated Cell Deposition Unit (ACDU) (Becton Dickinson & Co., Mountain View, CA).

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[1] *Abbreviations used in this paper:* ICAM-1, intercellular adhesion molecule 1; LDA, limiting dilution analysis; pcc, pigeon cytochrome c.

Isolation of Splenic B Cells. Sephadex G10 (Pharmacia LKB, Uppsala Sweden) nonadherent spleen cells were incubated with 3-1.5.5, RL172.4, J1j.10, SW3A4, DNase, and guinea pig C' (GIBCO BRL Gaithersburg, MD). Viable cells were collected over Lympholyte-M. For activation, cells were cultured in upright 25 cm^2 flasks (model 3050 Costar, Cambridge, MA) for 24–60 h at 5 × 10^6/ml with 10 μg/ml LPS B *Escherichia coli* 055:B5 (Difco Laboratories) or with 100 μg/ml poly I-poly C (Calbiochem-Novabiochem Corp., La Jolla, CA) in the absence or presence of 50 U/ml rIL-4. Purity of the resultant populations was determined by staining with FITC- or PE-B220 (αCD45R) (Pharmingen).

Isolation of Splenic Dendritic Cells. Splenic suspensions were incubated for 2 h in 5% CO_2 at 37°C on Falcon 3025 tissue culture plates (Becton Dickinson & Co., Cockeysville, MD). Nonadherent cells were removed and plates were incubated for another 6–24 h. Viable cells that had detached were collected after centrifugation over Lympholyte-M. Low density cells were further enriched by centrifugation over 50% Percoll (Pharmacia LKB, Piscataway, NJ). Purity of the resultant population was determined by staining with biotinylated 33D1 mAb followed by PE-streptavidin (Caltag Laborabories, South San Francisco, CA).

Limiting Dilution Analysis (LDA) of IL-2 Producers and APC. To determine IL-2 producer frequencies, CD4 T cells were titrated into 96-well round bottom plates (model 3799, Costar) with 20 replicate wells per cell density in the presence of excess peptide and APCs in a final volume of 50 μl. The APC frequency was determined by titrating APCs into wells containing nonlimiting numbers of CD4 T cells (i.e., 30–50). At least 72 h later, the plates were frozen, thawed, and supplemented with 10^3 CTL-L cells/well. After 16–24 h, the wells were pulsed with 1 μCi [^{3}H]thymidine (6.7 Ci/mmol; ICN Radiochemicals, Irvine, CA) and harvested 8–16 h later using a Brandell Cell Harvester (Medical Research and Development, Gaithersburg, MD). Proliferation of CTL-L, measured as isotope incorporation into DNA, was quantified with an LKB Betaplate scintillation counter (Wallac, Gaithersburg, MD). Background counts were determined in wells lacking CD4 cells, peptide, or APCs. Although CTL-L cells also proliferate in response to high concentrations of IL-4, the addition of αIL-4 mAb had no effect, whereas the addition of αIL-2 mAb prevented CTL-L growth, indicating that the only lymphokine measured in these cultures was IL-2. IL-2 concentrations in individual wells were determined by comparing CTL-L growth in these wells with growth in wells containing known quantities of rIL-2. The standard curve was fit to a logit function using a Marquand nonlinear regression algorithm. Wells were considered positive if the counts exceeded the mean background cpm by 3 SD. Statistical analyses were done on the logarithms of the IL-2 units as these data were more normally distributed than the counts per minute. Hence, the results are expressed as geometric means ×/÷ the standard error of these means. Occasional wells with values exceeding the maximum of the standard curve were excluded from the statistical analysis. Frequencies were determined by the χ^2 minimization method described by Taswell (23).

Results

CD4 T Cells Expressing Low Levels of CD44 as a Source of Naive T Cells. Our first task was to identify and isolate naive T cells. In immunized animals, CD44 is expressed at higher levels on primed T cells, which give detectable recall responses upon restimulation in vitro, than on naive T cells, which do not respond (24, 25). B10.A mice were immunized

with the pcc 81-104 peptide in CFA and tested after intervals of up to 3 mo. Lymph node and spleen cells were sorted for the expression of CD4 or additionally for the expression of Vα11 and various levels of CD44, the gates of which are shown in the inset to Fig. 1. IL-2–producing cells were detected among CD4 T cells taken from primed animals. Frequencies of 1/20 to 1/40 were observed with Vα11$^+$ CD44^{high} responders. In contrast, Vα11$^+$ cells expressing low or medium levels of CD44 contributed very little to the response. It should be noted that the frequency of responding cells was similar for unseparated CD4 T cells and for those further enriched in Vα11$^+$, CD44^{med} cells. Since CD4 T cells bearing Vα11-containing TCRs account for most of the response to the 81-104 fragment in B10.A mice (26) and represent 5% of the CD4 population (data not shown), we would have expected at least a 20-fold higher frequency among the CD44^{low} or CD44^{med} subsets if they were involved in the response. Therefore, the low frequencies observed in these subsets reflect either a small role for such cells in memory responses or a small contamination with CD44^{high} CD4 T cells. Similar results were observed in three independent experiments. Thus, most memory cells maintain high CD44 expression for long periods and would therefore be excluded from populations of naive cells selected by their CD44^{low} phenotype in young adult mice.

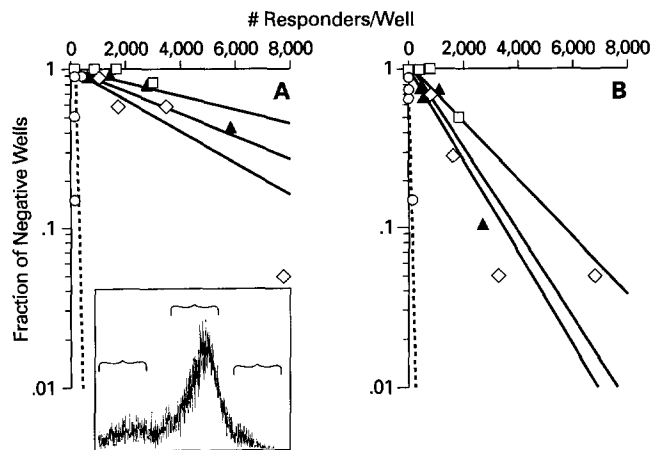*CD28 Ligation Costimulates IL-2 Production by Naive CD4$^+$ T Cells.* Memory CD4 Th1 clones fail to proliferate or produce IL-2 when triggered through their TCRs in the absence of costimulatory signals (27). Ligation of the CD28 molecule on the T cell suffices to costimulate IL-2 production and clonal expansion (28–30). To determine whether CD28 ligation could similarly costimulate naive T cells, we used CD4 T cells from animals transgenic for a TCR that recognizes the pcc 81-104 peptide presented by I-E^k. In transgenic mice of the appropriate MHC background, most of the peripheral T cells express the Vα11 transgene and CD4 (31) as well as low or medium levels of CD44; most CD44^{high} CD4 T cells do not express high levels of the Vα11 transgene (data not shown). To engage the TCR under noncostimulatory conditions, paraformaldehyde-fixed I-E^{k+} spleen cells were used to present the 81-104 peptide (27). Peripheral Vα11$^+$ CD44^{low} CD4 T cells produced IL-2 in response to peptide presented by irradiated splenic APCs as seen in Table 1, *Expt. 1*. Occasionally, the frequency of IL-2 producers was as high as 1/1. More typically the frequency was 1/2 to 1/5. In contrast, fixed spleen cells were unable to stimulate the CD4 T cells (frequency <1/20). IL-2 production could be restored, however, by the addition of αCD28 mAb. Similar results were observed in each of seven experiments. These data indicate that peptide-pulsed, fixed APCs are capable of triggering the TCR and that ligation of CD28 on naive CD4 T cells overcomes the costimulatory defect of fixed APCs.

Optimal Activation of Naive CD4 T Cells Requires Interaction with a CTLA-4 Binding Ligand on APCs. Costimulatory signals are delivered to T cells as a result of interactions between CD28 receptors on T cells and B7-1 and B7-2 ligands on APCs (29, 32). These ligands bind to CD28 (33) and more efficiently to CTLA-4, a receptor expressed on activated T cells (34, 35). We have used the solubilized receptor CTLA4Ig to determine whether costimulation of naive CD4 T cells involves a CTLA-4–reactive ligand(s) on APCs. Splenic APCs in the presence of the 81-104 peptide induced a high frequency of CD44^{low} transgenic CD4 T cells to produce IL-2 (Table 1, *Expt. 2*). In this and two additional experiments, the presence of the CTLA4Ig fusion protein reduced the responder frequency an average of 4.1-fold ($\times/\div$ 1.2). This effect was

Figure 1. Memory of CD4 T cells maintain high expression of CD44. (*Inset*) CD44 profile of Vα11$^+$ CD4 T cells from primed B10.A mice. Lymph node and spleen CD4 T cells ($\Diamond$) from mice primed 5 (*A*) or 12 (*B*) wk earlier with pcc 81-104 in CFA were further fractionated into Vα11$^+$ cells expressing low ($\square$), medium ($\blacktriangle$), or high ($\bigcirc$) levels of CD44 using the sort gates indicated in the inset. The cells were titrated into wells containing 10^5 irradiated (2,000 rad) T-depleted B10.A spleen cells in the presence of 30 μM 81-104. The IL-2 producer (responder) frequencies and 95% confidence intervals, calculated by χ^2 minimization, were: (*A*) 1/4,400 (3,300–5,300) ($\Diamond$), 1/10,300 (6,600–14,500) ($\square$), 1/6,300 (4,700–7,700) ($\blacktriangle$), and 1/41 (29-51) ($\bigcirc$); and (*B*) 1/1,700 (1,300–2,000) ($\Diamond$), 1/2,400 (1,800–3,000) ($\square$), 1/1,600 (1,200–1,800) ($\blacktriangle$), and 1/16 (12–20) ($\bigcirc$). No responses were detected with unprimed cells in the presence of peptide or with primed cells in the absence of peptide (data not shown).

Table 1. *Role of CTLA-4–reactive Ligands and CD28 in Activation of Naive CD4 T Cells*

Expt.	APC	CTLA4Ig	αCD28	Frequency
1	Irr	No	No	4.6 (3.8–5.1)
	Fix	No	No	>20 (undetectable)
	Fix	No	Yes	2.9 (2.3–3.4)
2	Irr	No	No	2.2 (1.6–2.6)
	Irr	Yes	No	6.4 (4.9–7.6)
	Irr	Yes	Yes	1.1 (0.9–1.2)

Reciprocal responder frequencies and 95% confidence intervals are shown as determined in the presence of 10 μM 81-104 and 2 $\times$ 10^4 2,000 rad irradiated (*Irr*) or paraformaldehyde-treated (*Fix*) T-depleted B10.A spleen cells in the absence or presence of 10 μg/ml mCTLA4Ig or αCD28 mAb (1:5,000 of ascites).

not observed with L6Ig, an irrelevant fusion protein of similar construction (36, and data not shown). Direct ligation of CD28 with αCD28 mAb overcame the inhibition seen with CTLA4Ig, restoring a high frequency of responding T cells. Thus, naive CD4 T cells become optimally activated only if their costimulatory receptor(s) engage a CTLA-4-reactive ligand. The failure to achieve >75% reduction in responding T cells even with saturating concentrations of mCTLA4Ig (10 μg/ml) raises the possibility that costimulatory molecules, in addition to those that bind CTLA-4, may participate in the activation of naive T cells.

Dendritic Cells and Activated B Cells as APCs for Naive CD4 T Cells. Because costimulation of IL-2 production by naive CD4 T cells depends on interactions with CTLA-4-reactive ligands on APCs and can be induced by CD28 ligation, one might predict that as long as an APC expressed the B7-1 or B7-2 ligands in addition to the appropriate peptide-MHC complex and adhesion molecules, it should be competent to activate naive T cells. Splenic dendritic cells isolated by adherence express one or more CTLA-4-reactive ligands, high levels of MHC class II molecules, and have been reported to be potent APCs for primary T cell responses (1, 2, 37). Activated B cells upregulate expression of B7-1 and B7-2, which confer comparable costimulatory function when transfected into COS cells (32). Therefore, both these types of APCs would be expected to stimulate naive CD4 T cells. Dendritic cells have been demonstrated, however, to be 10-30-fold more efficient than activated B cells as APCs (1), raising the possibility that previous reports attributing stimulatory function to B cell blasts actually reflect the APC function of contaminating dendritic cells in the B cell preparations. Therefore, to more definitively address the question of whether activated B cells can stimulate naive CD4 T cells, we cocultured the cells under conditions of limiting dilution. First, to be certain that any detected response was due to stimulation of naive CD4 T cells and not to potential memory T cell contaminants, we titrated CD44^{low} transgenic CD4 T cells into wells containing excess peptide and APC (Fig. 2). A high frequency of IL-2 producers was detected in cultures containing dendritic cells. As predicted, activated B cells also stimulated the CD4 T cells. Similar experiments indicated that poly I:C-activated CBA/J (I-E^k, Mls-1^a) B cells stimulated comparable frequencies of Mls-1^a-reactive CD44^{low} Vβ6$^+$ CD4 T cells from nontransgenic B10.A mice (data not shown). Careful quantitative analysis of 21 peptide presentation experiments indicated that B cell blasts stimulated a slightly, but statistically significantly (p <0.01) lower frequency of CD4 T cells. The average responder frequency detected with dendritic cells was 1/2.79 ($\times/\div$ 1.15) and with activated B cells was 1/4.59 ($\times/\div$ 1.16). Nonetheless, this 1.65-fold difference in frequency argues against the possibility that only contaminating memory T cells in the purified naive population were stimulated by activated B cells.

To rigorously demonstrate that activated B cells themselves, and not potentially contaminating dendritic cells, were responsible for stimulating IL-2 production, we next titrated the APCs in cultures with excess peptide and CD44^{low} trans-
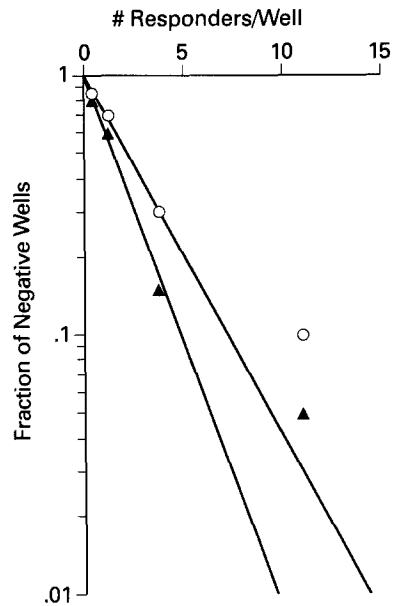

Figure 2. Activated B cells stimulate IL-2 production by naive CD4 T cells. CD44^{low} Vα11$^+$ transgenic CD4 T cells were titrated into wells containing 1 μM 81-104 and either 50 dendritic cells (O) or 500 LPS-activated B cells (▲) from B10.A mice. The responder frequencies were: 1/3.1 (2.2-3.9) (O) and 1/2.1 (1.5-2.0) (▲).

genic CD4 T cells. As shown in Fig. 3 *A*, the frequency of cells in the dendritic cell population capable of inducing IL-2 production by naive CD4 T cells was eightfold higher than the frequency of such APCs in the LPS-activated B cell preparation. The relative difference in APC frequency between these two cell types varied from 3-10-fold in several experiments, whether the B cells were activated with LPS, poly I:C, or
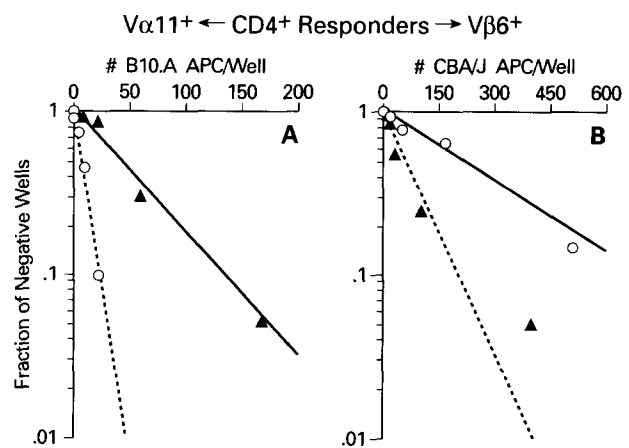

Figure 3. Frequencies of dendritic cells and activated B cells that stimulate IL-2 production by naive CD4 T cells. (*A*) Splenic dendritic cells (O) or LPS-activated B cells (▲) from B10.A mice were titrated into wells containing 1 μM 81-104 and 30 CD44^{low} Vα11$^+$ transgenic CD4 T cells. (*B*) Dendritic cells (O) or poly I:C-activated B cells (▲) from CBA/J mice were titrated into wells containing 30 CD44^{low} Vβ6$^+$ nontransgenic B10.A CD4 T cells. The APC frequencies were: (*A*) 1/7.0 (5.2-8.6) (O), 1/58 (42-73) (▲); and (*B*) 1/300 (215-370) (O), 1/86 (56-100) (▲).

anti-Ig (data not shown). Nonetheless, the B cell preparations were typically >97% B220$^+$ and were not contaminated with 33D1$^+$ cells (data not shown). Therefore, the stimulatory function detected in populations of activated B cells could not be readily attributed to contaminating non-B cells. To further establish this point, activated Mls-1^a-expressing B cells were used to stimulate IL-2 production by CD44^{low} V$_\beta$6$^+$ CD4 T cells from nontransgenic mice (Fig. 3 B). In this case, the expression of the TCR ligand is largely confined to B cells and CD8 T cells (38–40), which probably accounts for the low APC frequency detected among dendritic cells. The observation that dendritic cells stimulated at all could result from contaminating activated B cells as our dendritic cell preparations routinely contained 10–20% B220$^+$ cells. Thus, these results indicate that activated B cells can stimulate naive CD4 T cells to produce IL-2.

B Cell Blasts Elicit Less IL-2 than Do Dendritic Cells. With the exception of Mls-directed responses, activated B cells have been demonstrated to be inferior to dendritic cells in stimulating freshly isolated naive CD4 T cells (19) and mixtures of naive and memory T cells (1). This inferiority could partially be explained by our finding that the subset of cells capable of eliciting IL-2 production by naive CD4 T cells was smaller in populations of activated B cells than dendritic cells. We showed above, however, that the inferiority is evident even when the frequency of responding T cells was examined in the presence of excess presenting cells. Another possibility is that the potency of the cells within the stimulatory subsets of the two populations differed and determined the extent to which each T cell was stimulated. To examine this question, individual CD4 T cells were dispensed into wells containing excess peptide and either dendritic or activated B cells. The amount of IL-2 produced by each CD4 T cell was quantified and is presented in Fig. 4. In this experiment, the average amount of IL-2 produced by B cell–stimulated, responding CD4 T cells (0.0037 ×/÷ 1.13 U/cell) was 4.5-fold less than that produced by CD4 T cells stimulated with dendritic cells (0.0166 ×/÷ 1.23 U/cell). In four similar experiments, dendritic cells induced, on average, 3.70 ×/÷ 1.13-fold greater IL-2 production than did activated B cells (*p* <0.001).

To explore whether this difference had a trivial explanation, we first considered the possibility that the B cell blasts did not stimulate the T cells at all, but instead produced the IL-2 themselves. Freshly isolated lymph node B cells have been shown to express IL-2 mRNA (41). Similarly, splenic B cells stimulated in vitro secrete IL-2. Concentrations of FCS >2%, however, block this activity of B cells (42). Our cultures contained 10% FCS. Moreover, detection of IL-2 in these cultures depended on the presence of a single T cell. Therefore, we believe that T cells produced the IL-2 measured in our cultures. Second, B cell stimulated in vitro express the IL-2 receptor (43, 44). Thus, the detection of lower quantities of IL-2 in wells containing B blasts could be due to its consumption by the B cells. To test this, CTL-L growth in the standard IL-2 assay was examined in the presence of activated B cells. Although the amounts of IL-2 detected were
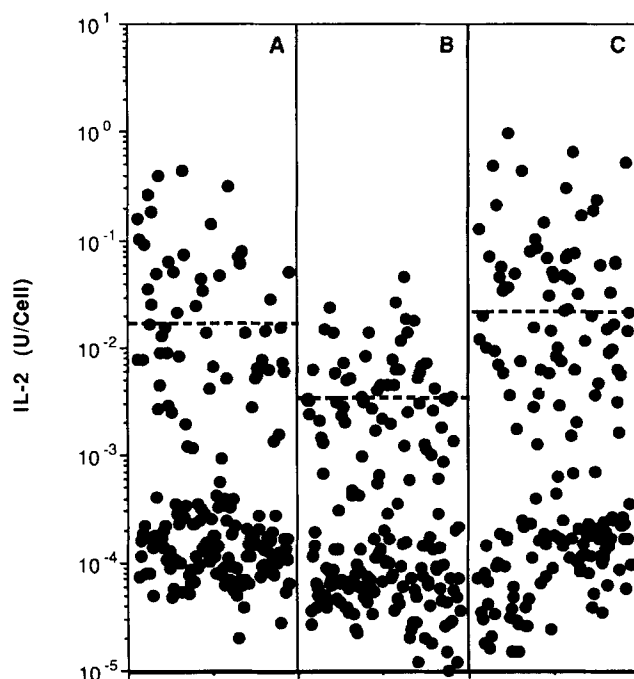

Figure 4. Naive CD4 T cells produce less IL-2 when stimulated by B cell blasts than by dendritic cells. Individual transgenic CD44^{low} Vα11$^+$ CD4 T cells were dispensed into wells containing 10 μM 81-104 and 10^3 B10.A dendritic cells (*A*), 10^3 poly I:C–activated B cells (*B*), or a mixture of 10^3 activated B cells and 10^3 dendritic cells (*C*). (*Dashed bars*) The geometric mean of the amount of IL-2 produced by a single T cell. Only positive wells (>3 SD above mean of control wells) were used in the calculation.

diminished by higher numbers of B cell blasts (10^4), the assay was not affected by the number of activated B cells used in our experiments (data not shown). Finally, activated B cells may produce factors that are toxic either to the responding CD4 T cells or the indicator CTL-L cells. To address this possibility, single CD4 T cells were deposited in wells containing either dendritic cells or B cell blasts alone or in combination. As shown in Fig. 4 C, the amount of IL-2 detected in wells containing the mixture of APC (0.0218 ×/÷ 1.21 U/ml) was not significantly different from that detected in wells with dendritic cell APC alone and was significantly higher than in wells with B cell APC alone (see above). Thus, activated B cells neither consumed appreciable quantities of IL-2 nor interfered with the activation of naive CD4 T cells by dendritic cells. We therefore conclude that even within the stimulatory subset, activated B cells are not as potent as dendritic cells in eliciting IL-2 production by naive CD4 T cells.

Failure to Detect Stimulatory Capacity from Every APC. The APC frequency detected among dendritic cell preparations in 27 experiments was on average 1/5.80 (×/÷ 1.09), and similar even when the cells were sorted for 33D1 expression (data not shown). A frequency of less than 1/1 might indicate that only a fraction of the cells provided both TCR occupancy and costimulatory signals. Because optimal IL-2

production required 72 h of culture, during which time there was extensive cell death among the APCs (data not shown), it is also possible that not all of the cells survived long enough to impart the necessary signals to the T cells. This possibility was explored by supplementing the cultures with trophic factors and determining the resultant effect on the detected APC frequencies. GM-CSF promotes survival of cultured murine epidermal Langerhans cells (45). Similarly, IL-4 improves viability of B cells stimulated with anti-IgM (46). Neither GM-CSF nor IL-4 alone had any costimulatory activity on freshly isolated naive CD4 T cells (data not shown). As seen in Fig. 5 A, ∼15% of the dendritic cells exhibited detectable APC function. Addition of IL-4 to the cultures increased this frequency only twofold. Supplementing the wells with GM-CSF, however, resulted in a 5.5-fold higher APC frequency; i.e., all the dendritic cells became capable of eliciting IL-2. This finding has been observed in five of six experiments with an average increase of 2.88 (×/÷ 1.23)-fold in the APC frequency. In parallel experiments, GM-CSF did not consistently affect APC frequencies in activated B cell preparations (Fig. 5 B). By contrast, in each of five trials, the presence of IL-4 in the cultures augmented activity of the B cells, with an average increase in the APC frequency of 4.33 (×/÷ 1.18)-fold. These results indicate that, depending on the conditions of culture, the calculated frequency of cells capable of stimulating naive CD4 T cells can be considerably underestimated. Moreover, the extent of underestimation could be greater for the population of activated B cells than for dendritic cells, because activated B cells exhibited poorer viability in culture (data not shown). Thus, it was possible that poorer survival of B cell blasts accounted for their inferior stimulation of IL-2 production by individual T cells (Fig. 4). To explore this possibility, the stimulatory potential of IL-4–sup-

plemented B cell blasts was assessed by measuring the amount of IL-2 they elicited from individual naive CD4 T cells. As seen in Fig. 6, IL-4 did not affect the maximum amount of IL-2 elicited by activated B Cells. In this experiment, the mean amount of IL-2 induced by activated B cells was 0.00052 ×/÷ 1.16 U/cell in the absence of IL-4 (Fig. 6 B) and 0.00038 ×/÷ 1.11 U/cell in its presence (Fig. 6 D). Thus, independent of their apparently lower APC frequency due to poor survival in culture, activated B cells failed to elicit as much IL-2 from naive CD4 T cells as did dendritic cells (0.0011 ×/÷ 1.19 U/cell, Fig. 6 A).

Nature of the Deficiency of Activated B Cells As APC. We next wished to determine the basis for the stimulatory deficiency of activated B cells. According to the two signal models of IL-2 induction in CD4 T cells, the lower potency of activated B cells could reflect deficiencies either in costimulation or antigen presentation. We compared the expression of potentially costimulatory CTLA-4–reactive molecules on dendritic cells and activated B cells. The histograms shown in Fig. 7 A demonstrate that both dendritic cells and activated B cells expressed high levels of CTLA-4–reactive ligands, although there was consistently threefold less on B cells than on dendritic cells. The functional role of these ligands was assessed by determining the frequencies of CD4 T cells stimulated by either type of APC in the absence or presence of CTLA4Ig (Fig. 7, B and C). The frequency of CD4 T cells induced to secrete IL-2 in the presence of CTLA4Ig dropped
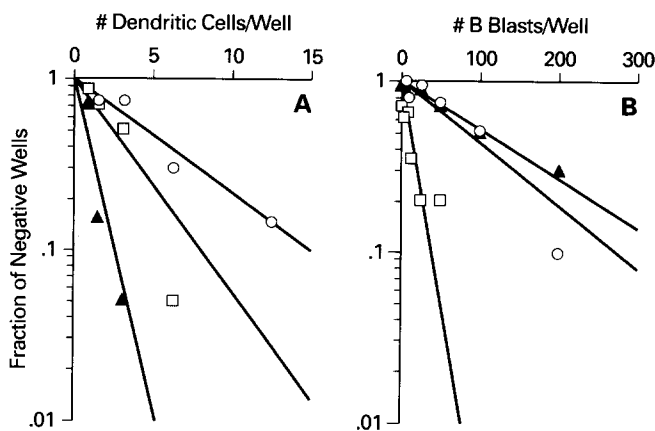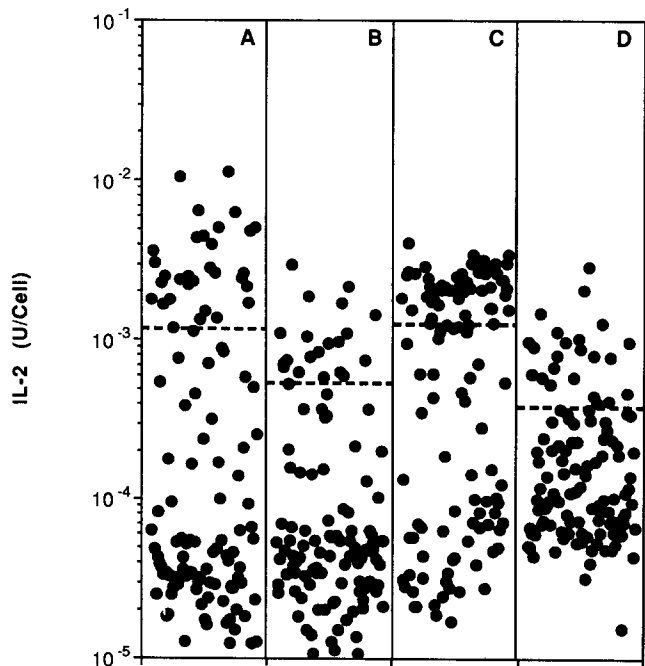

Figure 5. Lymphokines augment APC frequencies. Dendritic cells (A) or poly I:C–activated B cells (B) from B10.A mice were titrated into wells containing 50 CD44^low Vα11^+ transgenic CD4 T cells and 10 μM 81-104 in the absence (O) or presence of 10 ng/ml rmGM-CSF (▲) or 50 U/ml rIL-4 (□). APC frequencies were: (A) 1/6.6 (5.0–7.8) (O), 1/1.2 (0.9–1.5) (▲), 1/3.6 (2.7–4.3) (□); and (B) 1/122.5 (91.7–147.8) (O), 1/152 (115–182) (▲), 1/16.8 (14.4–18.3) (□).

Figure 6. CD28 ligation overcomes deficient APC function of B cell blasts. Individual CD44^low Vα11^+ CD4 cells were dispensed into wells containing 10 μM 81-104 and 10^3 B10.A dendritic cells (A) or 5 × 10^3 poly I:C–activated B cells in the absence (B) or presence of αCD28 mAb (C) or 50 U/ml IL-4 (D). Data are presented as in Fig. 4.
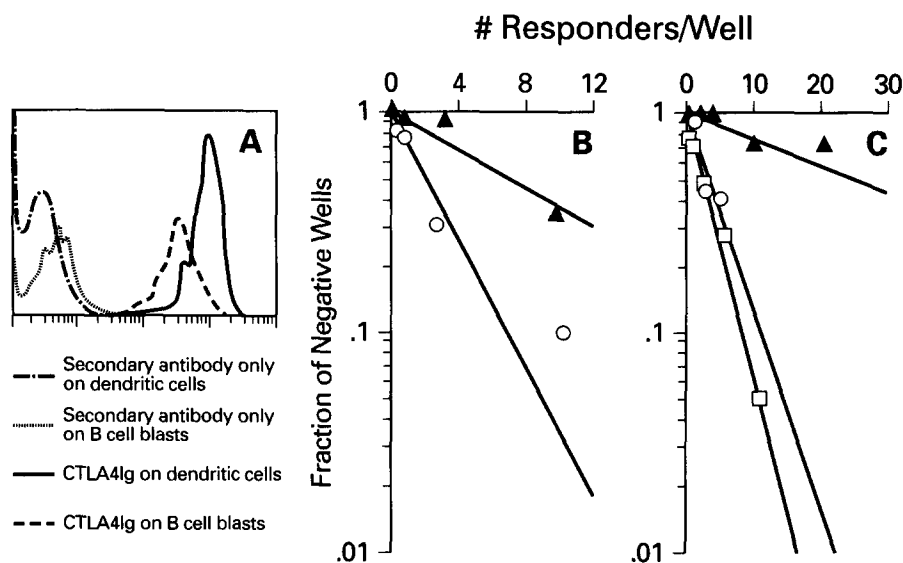
Responders/Well

Figure 7. Role for CTLA-4-binding ligands in APC function of dendritic cells and activated B cells. (*A*) Dendritic cells (_____, -·-) or poly I:C–activated B cells (- - -, ····) were incubated with 2.4G2, followed by hCTLA4Ig (_____, - - -) or buffer (-·; ····). All samples were further incubated with biotinylated mouse anti–human Ig, then PE-conjugated streptavidin. For the LDA, CD44^{low} Vα11$^+$ transgenic CD4 T cells were titrated into wells containing 81-104 and either 30 dendritic cells (*B*) or 200 poly I:C–activated B cells (*C*) from B10.A mice. Cells were cultured in medium alone (O), or were supplemented with 10 μg/ml CTLA4Ig (▲) or both CTLA4Ig and αCD28 (□). The responder frequencies were: (*B*) 1/3.4 (2.6–3.9) (O), 1/10.8 (7.6–13.7) (▲); and (*C*) 1/4.3 (3.3–5.2) (O), 1/35.1 (24.9–44.5) (▲), and 1/3.3 (2.6–3.8) (□).

in three experiments by an average of 3.07-fold ($\times/\div$ 1.43) when stimulated by dendritic cells and 11.45-fold ($\times/\div$ 1.14) in four experiments when stimulated by activated B cells. L6Ig, a control reagent, did not affect stimulation by either APC type (data not shown). Addition of αCD28 mAb completely overcame the CTLA4Ig-mediated inhibition of stimulation by B cell blasts (Fig. 7 *C*), suggesting that the CTLA4Ig effect was not nonspecific. Therefore, maximal APC function of both types of cells involves ligands with affinity for CTLA-4. In addition to the quantitative differences in expression of CTLA-4–reactive ligands, it is possible that the two APC populations differed qualitatively as well. The greater blocking effect of CTLA4Ig on B cell blasts than on dendritic cells suggests that dendritic cells possess alternative costimulatory ligands. Incomplete CTLA4Ig-mediated blocking of dendritic cell–stimulated primary alloresponses was similarly observed by Lenshow et al. (47). Therefore, it is possible that activated B cells are relatively inefficient at stimulating IL-2 production because they do not express sufficient levels of all the costimulatory molecules necessary for optimal activation of naive CD4 T cells.

To test this possibility, the ability of activated B cells to stimulate naive CD4 T cells was determined in the presence or absence of αCD28 mAb (Table 2). In the presence of αCD28, activated B cells exhibited a much higher APC frequency for peptide presentation than in the absence of the mAb. An augmented APC frequency in the presence of αCD28 mAb was also detected for activated B cells presenting Mls-1^a to Vβ6$^+$ CD4 T cells (data not shown). These findings indicate that the low frequency of APC within a population of activated B cells is due in part to a low frequency of cells with sufficient costimulatory function to activate naive CD4 T cells.

We also examined the amount of IL-2 produced by individual CD4 T cells stimulated by activated B cells in the absence or presence of αCD28 mAb. The addition of αCD28 mAb to the cultures containing B cells resulted in the production of larger quantities of IL-2 (mean = 0.0012 $\times/\div$ 1.12

U/cell, Fig. 6 *C* compared with 0.00052 $\times/\div$ 1.16 U/cell, Fig. 6 *B*), as high as those obtained in wells containing dendritic cells (mean = 0.0011 $\times/\div$ 1.19 U/ml, Fig. 6 *A*). This is one of seven similar experiments. Thus, the subset of activated B cells that can stimulate CD4 T cells does so less efficiently than dendritic cells, at least in part because of a deficiency in costimulation.

We next asked whether, in addition to being deficient in their ability to costimulate, activated B cells also provided suboptimal stimulation through the TCR. The expression of adhesion molecules is critical for optimal TCR-mediated activation (48, 49). Resting B cells express low levels of these molecules and have been characterized as having minimal APC function for unprimed T cells (37). In contrast, both dendritic cells and activated B cell express higher levels of adhesion molecules such as LFA-1 and ICAM-1, although the levels on activated B cells in our experiments were consistently two- to threefold lower than on dendritic cells (Fig. 8, *B* and *C*). Expression of I-E^k, the presenting element for the 81-104 peptide, was roughly equivalent on both cell types as shown in Fig. 8 *A*, and higher than the low levels reported on resting B cells (1). To examine the relative ability to engage the TCR, we compared the APC frequencies of dendritic cells and activated B cells at decreasing concentrations of the 81-104 peptide. αCD28 was included in the wells with B cells to ensure that costimulation was not limiting in these cultures. The results shown in Table 2 indicate that peptide became limiting for optimal antigen presentation at a threefold lower concentration for dendritic cells than for activated B cells in one experiment, and at the same concentration in a second experiment. By contrast, the ability of resting B cells to stimulate CD4 T cells was always more sensitive to decreases in peptide concentration (Table 2 and data not shown). We interpret these data to indicate that activated B cells are slightly deficient in expressing the required combination of adhesion molecules and peptide–MHC complexes to sufficiently engage the TCR and initiate the signal 1 pathway.
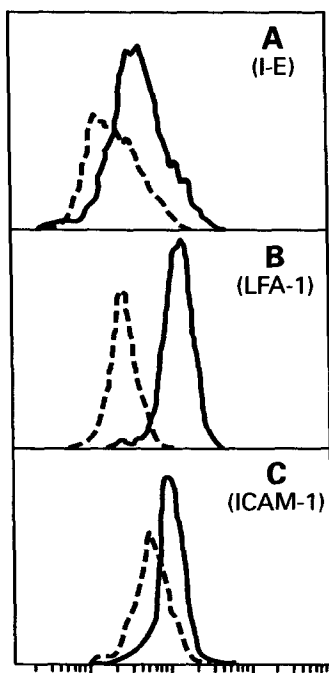
Figure 8. Relative expression of adhesion molecules. Dendritic cells (——) and poly I:C–activated B cells (- - -) were incubated with 2.4G2 followed by FITC-conjugated antibodies to I-E, LFA-1, and ICAM-1.

Discussion

Our understanding of the roles played by various APC in stimulating T cell immunity remains incomplete. Cells of the Langerhans/dendritic cell lineage take up, process, and present soluble protein (50–53) and have been implicated in the initial capture of antigen and presentation of antigen-derived peptides (54). Moreover, because dendritic cells stimulate freshly explanted T cells from unmanipulated animals more efficiently than do B cells or macrophages (1, 55), the idea that dendritic cells are the initiating APC in immune responses has gained acceptance (15, 17).

There are conflicting reports concerning the ability of ac-

tivated B cells to stimulate unprimed T cells. In support of such a role, optimal T cell priming was found to require B cells. Thus, lymph node T cells from B cell–depleted mice injected with protein antigen in CFA proliferated poorly when challenged with antigen in vitro (12–16). T cell priming did occur, however, when haptenated antigen in CFA was administered together with hapten-primed B cells (15). Lin et al. (17) have proposed a model in which dendritic cells prime the first cohort of native T cells, which then provide help for activation of B cells. Once activated, the B cells can then directly prime additional naive T cells (16, 17). In this report, however, it was not shown that activated B cells directly stimulated naive T cells. Instead, the activated B cells may have produced antibody that formed complexes with the antigen, and the initiating APC may have been a non-B cell capable of taking up the complexes and presenting antigenic fragments (10).

There are two recent reports that contradict the notion that activated B cells stimulate naive T cells. In the first case, Fuchs and Matzinger (22) demonstrated that injection into naive mice of activated B cells, unlike unfractionated spleen cells, failed to prime and instead tolerized CD8 CTL precursors (CTLp) specific for the H-Y antigen expressed by the injected cells. In the second study, Ronchese and Hausmann (21) transferred HSA^{low} thymocytes into SCID mice either alone or with antigen-primed B cells. Injection of antigen into both sets of reconstituted recipients primed only those T cells restricted to the MHC of the non-B APC. The authors interpreted these results to mean that activated B cells could not stimulate naive T cells (21).

Given the controversy with in vivo experiments and the failure of in vitro studies to demonstrate beyond doubt that activated B cells, rather than non-B contaminants, stimulate naive T cells, we decided to reexamine this question. As a convenient source of naive T cells, we used CD4^{low}, Vα11$^+$ cells from TCR transgenic mice. To assure ourselves that the

Table 2. *Relative Efficiency of Peptide Presentation by Different APCs*

Expt.	(81-104)	PIC B*	PIC B plus αCD28	Dendritic cells	Dense B cells
1	1.0	47.4 (39.6–52.7)	9.3 (8.2–10.0)	6.8 (5.3–8.0)	
	0.1		10.5 (8.9–11.6)	16.8 (13.1–19.6)	
	0.01		31.0 (23.8–36.7)	27.8 (21.7–32.3)	
2	10.0	122 (92–148)	4.4 (3.3–5.3)		
	1.0		8.0 (6.1–9.5)	5.6 (4.1–6.7)	6.5 (5.2–7.5)
	0.33		8.5 (6.7–9.8)	5.6 (4.4–6.5)	10.5 (7.9–12.6)
	0.11		13.5 (10.1–16.3)	9.1 (6.9–10.8)	14.5 (11.1–7.2)
	0.037		25.8 (19.1–31.3)	11.7 (8.7–14.2)	74.1 (55.4–9.5)
	0.012		66.7 (49.4–81.3)	25.9 (19.2–31.4)	378 (254–505)

Reciprocal APC frequencies and 95% confidence intervals are shown as determined in the presence of CD44^{low} transgenic CD4 T cells, the absence or presence of αCD28 mAb, and the indicated micromolar concentrations of peptide 81-104.
* Poly I:C-activated B cells.

activation requirements of these cells reflected those of naive cells developing in a normal animal, we included in our study Mls-reactive CD44^{low}, Vβ6$^+$ CD4 T cells isolated from nontransgenic mice. IL-2 production by these naive CD4 T cells required costimulatory signals that could be provided by CD28 ligation and that depended on interaction with CTLA-4–reactive ligands expressed by APC. Since both dendritic cells and B cell blasts express such ligands and since both B7-1 and B7-2 have costimulatory function (35, 56), both APC types were expected to stimulate naive CD4 T cells. Indeed, we found that the frequency of naive CD4 T cells capable of responding either to 81-104/E^k or to Mls presented by activated B cells was high, although slightly lower than the frequency of naive T cells responding to 81-104/E^k presented by dendritic cells. This was not evident from previous studies. Using primary MLR against alloantigens other than Mls, Metlay et al. (1) reported that 10–30 times higher numbers of B cell blasts were required to stimulate responses equivalent to those induced by dendritic cells. Our results in nonlymphokine-supplemented medium, where the APC survival is poor, confirm this finding. Metlay et al. (37) noted that the reduced potency of activated B cells could mean that B cell blasts are efficient APC only for a subset of T cells or that B cell blasts stimulate all T cells but only 3–10% as well as dendritic cells. A third possibility is that only a small subset of activated B cells has detectable APC function. Our results strongly support the second and third possibilities, and are compatible with a small contribution from the first. In addition, the frequency of B cells capable of eliciting IL-2 production by naive transgenic CD4 T cells was high enough, especially when cultured with IL-4, to rule out the possibility that the observed stimulating capacity was due to contaminating dendritic cells or any other non-B cell. Detection of a higher frequency of Mls-presenting APC among activated B cells than among dendritic cells provides particularly compelling evidence that B cells can stimulate naive CD4 T cells.

Because of poor cell survival in vitro, the stimulatory deficiency of activated B cells may not have been reliably indicated by the responder and APC frequencies we detected in the cultures. The single cell experiments, however, revealed that the extent to which T cells were activated to produce IL-2 was fourfold lower after stimulation with B cell blasts than with dendritic cells. Metlay et al. (37) hold the view that the relative inability of B cell blasts to form aggregates with T cells accounts for the inferior stimulatory function of activated B cells. These investigators have documented a role for LFA-1 in the antigen-dependent interaction between T cells and both APC types (55), and suggest that dendritic cells, but not B blasts, have additional clustering capabilities that are independent of LFA-1 and antigen (37). We found that B cell blasts expressed slightly lower levels of LFA-1 and ICAM-1 and that their APC function was, in some cases, more sensitive to decreases in peptide concentration than was seen with dendritic cells. Although our data as well as that of Jenkins et al. (57) indicate that potential differences in LFA-1–dependent T-APC interactions do not always exert a de-

tectable effect on TCR occupancy for either naive or memory T cells, it is possible that the superior clustering function of dendritic cells serves to facilitate delivery of costimulatory signals. Consistent with this, we found with nonlimiting peptide concentrations that the relative stimulatory deficiency of the B cells was overcome by directly ligating the CD28 molecule on the CD4 T cell. Thus, activated B cells are also poorer APC because they fail to provide adequate costimulatory signals to naive CD4 T cells. This costimulatory deficiency of activated B cells has recently been observed for stimulation of certain Th1 clones as well (58).

Our studies show that naive peripheral CD4 T cells stimulated by B cell blasts produce only 25% the amount of IL-2 elicited by dendritic cells. This observation might help to reconcile some of the conflicting reports in the literature. In the experiments of Lin et al. (17), where activated B cells are thought to play a role in T cell priming, the antigen was administered in CFA. This may have eliminated the costimulation deficiency of the primed B cells and allowed them to present antigen to naive T cells. In the SCID reconstitution experiments of Ronchese and Hausmann (21), it was noted that T cells restricted to the MHC of cotransferred B cells were primed when splenic rather than thymic CD4 T cells were transferred into the mice. The authors believe this was due to contaminating antigen-experienced T cells in the transferred splenic populations. Another possibility, however, is that the inferiority of activated B cells may be more pronounced with thymocytes than it is with naive peripheral T cells. In the study of Fuchs and Matzinger, (22) H-Y specific CTLp were tolerized after recognition of antigen on activated B cells. CTL priming often requires activation of CD4 T cells to produce IL-2 and perhaps other factors as well (59–62), in the absence of which the CTL become tolerized (63, 64). In preliminary experiments, the frequency of IL-2 producers detected in H-Y tolerant animals was higher than that seen in unprimed mice, but two- to threefold lower than that seen in primed animals (Schwartz, R. H. and C. Chen, unpublished observations). This suggests that the CD4 population was affected. It is not known how much IL-2 a CD4 T cell must produce either to support its own clonal expansion or to provide help in the activation of other T cells. Whether a fourfold reduction in lymphokine production is large enough to account for the CTL tolerance or lack of thymocyte priming reported in vivo is unclear. A three- to fourfold difference in production in vivo of other Th1 lymphokines, however, is associated with a dramatic physiologic difference in susceptibility to certain bacterial infections (65).

The major finding of the current study is that, in spite of their high levels of CTLA-4/CD28–reactive ligands, activated B cells are quantitatively deficient in providing costimulatory signals necessary for IL-2 production by naive CD4 T cells. To some extent, the costimulatory ligands involved in the APC function of both dendritic cells and activated B cells appear to be similar since CTLA4Ig impaired stimulation of T cells in response to either APC. The relative roles of the currently known murine CTLA-4 ligands, B7-1 and B7-2, on the two APC types is not entirely clear. B7-1 has been

reported to represent the major costimulatory ligand on some but not all APC capable of delivering such signals (57, 66). Activated B cells express relatively low levels of B7-1 and αB7-1 mAb does not effectively block their APC function (35), implying that B7-1 plays a minor role in the stimulatory activity of B cell blasts. Murine splenic dendritic cells and cultured Langerhans cells have been shown to transcribe and express B7-1 (48, 67). αB7-1 mAb also fails to fully block stimulation of human T cells by allogeneic dendritic cells (66). In contrast to B7-1, B7-2 is highly expressed by B cell blasts and represents the primary CTLA-4–reactive ligand on these

cells (35). Moreover, B7-2 appears to be the major costimulatory molecule on activated B cells because αB7-2 mAb blocks their APC function (35). It is interesting to note that we have found, in preliminary experiments, that whereas αB7-2 mAb readily blocks IL-2 production by naive CD4 T cells induced by activated B cells, it has no effect on the response elicited by dendritic cells (our unpublished observations). Because dendritic cell–induced IL-2 production is incompletely blocked by CTLA4Ig, αB7-1, and αB7-2 mAb, there may be as yet undefined costimulatory ligands important to the unique function of these cells.

References

1. Metlay, J.P., E. Puré, and R.M. Steinman. 1989. Distinct features of dendritic cells and anti-Ig activated B cells as stimulators of the primary mixed leukocyte reaction. *J. Exp. Med.* 169:239.
2. Liu, Y., B. Jones, W. Brady, C.A. Janeway, Jr., and P.S. Linsley. 1992. Co-stimulation of murine CD4 T cell growth: cooperation between B7 and heat-stable antigen. *Eur. J. Immunol.* 22:2855.
3. Krieger, J.I., R.W. Chesnut, and H.M. Grey. 1986. Capacity of B cells to function as stimulators of a primary mixed leukocyte reaction. *J. Immunol.* 137:3117.
4. Lanzavecchia, A. 1985. Antigen-specific interactions between T and B cells. *Nature (Lond.).* 314:537.
5. Ozaki, S., and J.A. Berzofsky. 1987. Antibody conjugates mimic specific B cell presentation of antigen: relationship between T and B cell specificity. *J. Immunol.* 138:4133.
6. Manca, F., D. Fenoglio, A. Kunk, C. Cambiaggi, M. Sasso, and F. Celada. 1988. Differential activation of T cell clones stimulated by macrophages exposed to antigen complexed with monoclonal antibodies. A possible influence of paratope specificity on the mode of antigen processing. *J. Immunol.* 140:2893.
7. Watts, C., and A. Lanzavecchia. 1993. Suppressive effect of antibody on processing of T cell epitopes. *J. Exp. Med.* 178:1459.
8. Mamula, M.J., and C.A. Janeway, Jr. 1993. Do B cells drive the diversification of immune responses? *Immunol. Today.* 14:151.
9. Lehmann, P.V., T. Forthuber, A. Miller, and E. Sercarz. 1992. Spreading of T-cell autoimmunity to cryptic determinants of an auto antigen. *Nature (Lond.).* 358:155.
10. Fuchs, E., and P. Matzinger. 1993. B cells drive diversification—but not directly. *Immunol. Today.* 14:153.
11. Etlinger, H.M., D. Gillessen, H.-W. Lahm, H. Matile, H.-J. Schonfeld, and A. Trzeciak. 1990. Use of prior vaccinations for the development of new vaccines. *Science (Wash. DC).* 249:423.
12. Ron, Y., P. DeBastelier, E. Tzevohal, J. Gordon, M. Feldman, and S. Segal. 1983. Defective induction of antigen-reactive proliferating T cells in B cell–depleted mice. *Eur. J. Immunol.* 13:167.
13. Hayglass, K.T., S.J. Naides, C.F. Scott, Jr., B. Benacerraf, and M.-S. Sy. 1986. T cell development in B cell-deficient mice. IV. The role of B cells as antigen presenting cells in vivo. *J. Immunol.* 136:823.
14. Janeway, C.A., Jr., J. Ron, and M.E. Katz. 1987. The B cell is the initiating antigen-presenting cell in peripheral lymph nodes. *J. Immunol.* 138:1051.
15. Ron, Y., and J. Sprent. 1987. T cell priming in vivo: a major role for B cells in presenting antigen to T cells in lymph nodes. *J. Immunol.* 138:2848.
16. Kurt-Jones, E.A., D. Liano, K.A. Hayglass, B. Benacerraf, M.-S. Sy, and A.K. Abbas. 1988. The role of antigen-presenting B cells in T cell priming in vivo. Studies of B cell–deficient mice. *J. Immunol.* 140:3773.
17. Lin, R.-H., M.J. Mamula, J.A. Hardin, and C.A. Janeway, Jr. 1991. Induction of autoreactive B cells allows priming of autoreactive T cells. *J. Exp. Med.* 173:1433.

18. Liu, Y., and C.A. Janeway, Jr. 1991. Microbial induction of co-stimulatory activity for CD4 T-cell growth. *Int. Immunol.* 3:323.

19. Croft, M., D.D. Duncan, and S.L. Swain. 1992. Response of naive antigen-specific CD4^{+} T cells in vitro: characteristics and antigen-presenting cell requirements. *J. Exp. Med.* 176:1431.

20. Bell, E.B., and S.M. Sparshott. 1990. Interconversion of CD45R subsets of CD4 T cells in vivo. *Nature (Lond.).* 348:163.

21. Ronchese, F., and B. Hausmann. 1993. B lymphocytes in vivo fail to prime naive T cells but can stimulate antigen-experienced T lymphocytes. *J. Exp. Med.* 177:679.

22. Fuchs, E.J., and P. Matzinger. 1992. B cells turn off virgin but not memory T cells. *Science (Wash. DC).* 258:1156.

23. Taswell, C. 1981. Limiting dilution assays for the determination of immunocompetent cell frequencies. I. Data analysis. *J. Immunol.* 126:1614.

24. Budd, R.C., J.-C. Cerottini, C. Horvath, C. Bron, T. Pedrazzini, R.C. Howe, and H.R. MacDonald. 1987. Distinction of virgin and memory T lymphocytes. Stable acquisition of the Pgp-1 glycoprotein concomitant with antigenic stimulation. *J. Immunol.* 138:3120.

25. Butterfield, K., C.G. Fathman, and R.C. Budd. 1989. A subset of memory CD4^{+} helper T lymphocytes identified by expression of Pgp-1. *J. Exp. Med.* 169:1461.

26. Fink, P.J., L.A. Matis, D.L. McElligott, M. Bookman, and S.M. Hedrick. 1986. Correlations between T-cell specificity and the structure of the antigen receptor. *Nature (Lond.).* 321:219.

27. Jenkins, M.K., and R.H. Schwartz. 1987. Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. *J. Exp. Med.* 165:302.

28. Thompson, C.B., T. Lindsten, J.A. Ledbetter, S.L. Kunkel, H.A. Young, S.G. Emerson, J.M. Leiden, and C.H. June. 1989. CD28 activation pathway regulates the production of multiple T-cell derived lymphokines/cytokines. *Proc. Natl. Acad. Sci. USA.* 86:1333.

29. Linsley, P.S., W. Brady, L. Grosmaire, A. Aruffo, N.K. Damle, and J.A. Ledbetter. 1991. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. *J. Exp. Med.* 173:721.

30. Harding, F.A., J.G. McArthur, J.A. Gross, D.H. Raulet, and J. Allison. 1992. CD28-mediated signalling costimulates murine T cells and prevents induction of anergy in T cell clones. *Nature (Lond.).* 356:607.

31. Kaye, J., M.-L. Hsu, M.-E. Sauron, S.C. Jameson, N.R.J. Gascoigne, and S.M. Hedrick. 1989. Selective development of CD4^{+} T cells in transgenic mice expressing a class II MHC-restricted antigen receptor. *Nature (Lond.).* 341:746.

32. Freeman, G.J., J.G. Gribben, V.A. Boussiotis, J.W. Ng, V.A. Restivo, Jr., L.A. Lombard, G.S. Gray, and L.M. Nadler. 1993. Cloning of B7-2: a CTLA-4 counter-receptor that costimulates human T cell proliferation. *Science (Wash. DC).* 262:909.

33. Linsley, P.S., E.A. Clark, and J.A. Ledbetter. 1990. T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. *Proc. Natl. Acad. Sci. USA.* 87:5031.

34. Linsley, P.S., W. Brady, M. Urnes, L.S. Grosmaire, N.K. Damle, and J.A. Ledbetter. 1991. CTLA-4 is a second receptor for the B cell activation antigen B7. *J. Exp. Med.* 174:561.

35. Hathcock, K.S., G. Laszlo, H.B. Dickler, J. Bradshaw, P. Linsley, and R.J. Hodes. 1993. Identification of an alternative CTLA-4 ligand costimulatory for T cell activation. *Science (Wash. DC).* 262:905.

36. Fell, H.P., M.A. Gaylel, D. Yelton, L. Lipsich, G.L. Schieven, J.J. Marken, A. Aruggo, K.E. Hellstrom, I. Hellstrom, and J. Bajorath. 1992. Chimeric L6 anti-tumor antibody. Genomic construction, expression, and characterization of the antigen binding site. *J. Biol. Chem.* 267:15552.

37. Metlay, J.P., E. Pure, and R.M. Steinman. 1989. Control of the immune response at the level of antigen-presenting cells: a comparison of the function of dendritic cells and B lymphocytes. *Adv. Immunol.* 47:45.

38. Molina, I.J., N.A. Cannon, R. Hyman, and B.T. Huber. 1989. Macrophages and T cells do not express Mls[a] determinants. *J. Immunol.* 143:39.

39. Webb, S.R., A. Okamoto, Y. Ron, and J. Sprent. 1989. Restricted tissue distribution of Mls[a] determinants. Stimulation of Mls[a]-reactive T cells by B cells but not by dendritic cells or macrophages. *J. Exp. Med.* 169:1.

40. Webb, S.R., and J. Sprent. 1990. Induction of neonatal tolerance to Mls[a] antigens by CD8^{+} T cells. *Science (Wash. DC).* 248:1643.

41. Heinzel, F.P., M.D. Sadick, S.S. Mutha, and R.M. Locksley. 1991. Production of interferon γ, interleukin 2, interleukin 4, and interleukin 10 by CD4^{+} lymphocytes *in vivo* during healing and progressive murine leishmaniasis. *Proc. Natl. Acad. Sci. USA.* 88:7011.

42. Taira, S., M. Matsui, K. Hayakawa, T. Yokoyama, and H. Nariuchi. 1987. Interleukin secretion by B cell lines and splenic B cells stimulated with calcium ionophore and phorbol ester. *J. Immunol.* 139:2957.

43. Malek, T.R., R.J. Robb, and E.M. Shevach. 1983. Identification and initial characterization of a rat monoclonal antibody reactive with the murine interleukin 2 receptor–ligand complex. *Proc. Natl. Acad. Sci. USA.* 80:5694.

44. Zubler, R.H., J.W. Lowenthal, F. Erard, N. Hashimoto, R. Devos, and H.R. MacDonald. 1984. Activated B cells express receptors for, and proliferate in response to, pure interleukin 2. *J. Exp. Med.* 160:1170.

45. Witmer-Pack, M.D., W. Olivier, J. Valinsky, G. Schuler, and R.M. Steinman. 1987. Granulocyte/macrophage colony-stimulating factor is essential for the viability and function of cultured murine epidermal Langerhans cells. *J. Exp. Med.* 166:1484.

46. Hodgkin, P.D., N.F. Go, J.E. Cupp, and M. Howard. 1991. Interleukin-4 enhances anti-IgM stimulation of B cells by improving cell viability and by increasing the sensitivity of B cells to the anti-IgM signal. *Cell. Immunol.* 134:14.

47. Lenshow, D.J., G.H.-T. Su, L.A. Zuckerman, N. Nabavi, C.L. Jellis, G.S. Gray, J. Miller, and J.A. Bluestone. 1993. Expression and functional significance of an additional ligand for CTLA-4. *Proc. Natl. Acad. Sci. USA.* 90:11054.

48. Geppert, T.D., L.S. Davis, H. Gur, M.C. Wacholtz, and P.E. Lipsky. 1990. Accessory cell signals involved in T cell activation. *Immunol. Rev.* 117:5.

49. van Seventer, G.A., Y. Shimizu, and S. Shaw. 1991. Roles of multiple accessory molecules in T-cell activation. *Curr. Opin. Immunol.* 3:294.

50. Levine, T.P., and B.M. Chain. 1992. Endocytosis by antigen presenting cells: dendritic cells are as endocytically active as other antigen presenting cells. *Proc. Natl. Acad. Sci. USA.* 89:8342.

51. De Bruijn, M.L.H., J.D. Nieland, C.V. Harding, and C.J.M. Melief. 1992. Processing and presentation of intact hen egg white lysozyme by dendritic cells. *Eur. J. Immunol.* 22:2347.

52. Inaba, K., J.P. Metlay, M.T. Crowley, and R.M. Steinman. 1990. Dendritic cells pulsed with protein antigens in vitro can prime antigen-specific MHC-restricted T cells in situ. *J. Exp.*

Med. 172:631.

53. Sornasse, T., V. Flamand, G. De Becker, H. Bazin, F. Tielemans, K. Thielmans, J. Urbain, O. Leo, and M. Moser. 1992. Antigen-pulsed dendritic cells can efficiently induce an antibody response in vivo. *J. Exp. Med.* 175:15.

54. Crowley, M., K. Inaba, and R.M. Steinman. 1990. Dendritic cells are the principal cells in mouse spleen bearing immunogenic fragments of foreign proteins. *J. Exp. Med.* 172:383.

55. Inaba, K., and R.M. Steinman. 1984. Resting and sensitized T lymphocytes exhibit distinct stimulatory (antigen-presenting cell) requirements for growth and lymphokine release. *J. Exp. Med.* 160:1717.

56. Razi-Wolf, Z., G.J. Freeman, F. Galvin, B. Benacerraf, L. Nadler, and H. Reiser. 1992. Expression and function of the murine B7 antigen, the major costimulatory molecule expressed by peritoneal exudate cells. *Proc. Natl. Acad. Sci. USA.* 89:4210.

57. Jenkins, M., E. Burrell, and J.D. Ashwell. 1990. Antigen presentation by resting B cells. Effectiveness at inducing T cell proliferation is determined by costimulatory signals, not T cell receptor occupancy. *J. Immunol.* 144:1585.

58. Gilbert, K.M., and W.O. Weigle. 1994. Tolerogenicity of resting and activated B cells. *J. Exp. Med.* 179:249.

59. Keene, J.A., and J. Forman. 1982. Helper activity is required for the in vivo generation of cytotoxic T lymphocytes. *J. Exp. Med.* 155:768.

60. Cassell, D., and J. Forman. 1988. Linked recognition of helper and cytotoxic antigenic determinants for the generation of cyto-

toxic T lymphocytes. *Ann. N.Y. Acad. Sci.* 532:51.

61. Widmer, M.B., and K.H. Grabstein. 1987. Regulation of cytolytic T-lymphocyte generation by B-cell stimulatory factor. *Nature (Lond.).* 326:795.

62. Kundig, T.M., H. Schorle, M.F. Bachmann, H. Hengartner, R.M. Zinkernagel, and I. Horak. 1993. Immune responses in interleukin-2-deficient mice. *Science (Wash. DC).* 262:1059.

63. Guerder, S., and P. Matzinger. 1989. Activation versus tolerance: a decision made by T helper cells. *Cold Spring Harbor Symp. Quant. Biol.* 54:799.

64. Rees, M.A., A.S. Rosenberg, T.I. Munitz, and A. Singer. 1990. In vivo induction of antigen-specific transplantation tolerance to Qa-1^a by exposure to alloantigen in the absence of T-cell help. *Proc. Natl. Acad. Sci. USA.* 87:2765.

65. Iizawa, Y., R.D. Wagner, and C.J. Czuprynski. 1993. Analysis of cytokine mRNA expression in *Listeria*-resistant C57BL/6 and *Listeria*-susceptible A/J mice during *Listeria monocytogenes* infection. *Infect. Immun.* 61:3739.

66. Young, J.W., L. Koulova, S.A. Soergel, E.A. Clark, R.M. Steinman, and B. Dupont. 1992. The B7/BB-1 antigen provides one of several costimulatory signals for the activation of CD4$^+$ T lymphocytes by human blood dendritic cells in vitro. *J. Clin. Invest.* 90:229.

67. Larsen, C.P., S.C. Ritchie, T.C. Pearson, P.S. Linsley, and R.P. Lowry. 1992. Functional expression of the costimulatory molecule, B7/BB1 on murine dendritic cell populations. *J. Exp. Med.* 176:1215.