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Intercellular adhesion molecule-1 inhibits interleukin 4 production by naive T cells

(cytokines/B7/*Drosophila* antigen-presenting cell)

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ABSTRACT **The type of cytokines produced during T cell responses determines susceptibility or resistance to many pathogens and influences the development of autoimmunity and allergy. To define the role of individual accessory molecules in cytokine production during primary immune responses, *Drosophila* cell lines expressing murine major histocompatibility complex class II molecules with defined combinations of accessory molecules were used to present peptide antigen to naive T cell receptor transgenic T cells. Significantly, expression of B7.1 or B7.2 without additional accessory molecules led to very high production of interleukin (IL)-4, which contrasted with minimal IL-4 production elicited by conventional antigen presenting cells (APC). However, coexpression of ICAM-1 and B7 on *Drosophila* APC induced little IL-4, suggesting an inhibitory role for intercellular adhesion molecule-1 (ICAM-1). In support of this idea, stimulation of T cell receptor transgenic T cells with peptide presented by splenic APC devoid of ICAM-1 (from ICAM-1-deficient mice) led to high IL-4 production. Thus, the level of IL-4 production by naive CD4$^+$ T cells during typical primary responses appears to be controlled, at least in part, by T–APC interactions involving ICAM-1.**

Effector CD4$^+$ T cells can be categorized on the basis of the cytokines produced after activation as Th1 cells producing interleukin 2 (IL-2), interferon γ (IFN-γ), and lymphotoxin, and T helper 2 (Th2) cells producing predominantly IL-4, IL-5, and IL-10 (1). The cytokines produced during immune responses determine the susceptibility of the host to a variety of pathogenic infections (e.g., Leishmania and parasitic gastrointestinal nematodes) (2–4). Cytokines are also known to influence the host's vulnerability to autoimmune disease and allergies such as asthma (5–8). Thus, defining the factors that influence cytokine production is clearly of considerable importance.

Certain cytokines play a well-documented role in determining the particular spectrum of cytokines secreted by T cells (9–12). For example, IL-12 and IFN-γ direct T cell differentiation into Th1 cells, whereas IL-4 controls the production of Th2 cells. In addition, the type of accessory molecules engaged (e.g., B7.1 vs. B7.2) (13, 14) has been reported to influence the development of effector T cells producing different patterns of cytokines.

Determining the influence of individual accessory molecules on cytokine production is complicated by the fact that most cell types express a wide variety of cell surface and secreted molecules with potential costimulatory or adhesive function. To simplify the analysis of the role of individual accessory molecule interactions on CD4$^+$ T cell responses, we used a system involving the expression of defined mammalian accessory molecules in nonmammalian cells, namely in a *Drosophila* cell line. These cells appear to provide a neutral background for the expression of defined accessory molecules and when transfected with major histocompatibility complex (MHC) molecules, act as potent APC for naïve T cells. The absence of typical antigen-presenting cell (APC)-derived cytokines such as IL-12, permit analysis of accessory molecule-mediated effects on T cell function in the absence of the well-known modulating influences of these cytokines. The efficacy of this approach has been well established in studies examining the activation requirements for CD8$^+$ T cells by using class I transfected *Drosophila* APC (15). Our studies demonstrate a previously undescribed role for intercellular adhesion molecule-1 (ICAM-1) in modifying the type of cytokines produced in primary CD4$^+$ T cell responses. Whereas B7-mediated costimulation promotes production of IL-4 and IL-10 by naive CD4$^+$ cells as previously suggested (16), coexpression of ICAM-1 and B7 on APC down-regulates these Th2 type cytokines.

MATERIALS AND METHODS

Animals. D011 T cell receptor (TCR) transgenic mice were obtained from D. Lo and K. Murphy (Washington University School of Medicine, St. Louis, MO). These mice were backcrossed to C57BL/6J-Icam/tm/Bay mice (17) from The Jackson Laboratories to generate ICAM-1-deficient APC.

Generation of *Drosophila* APC. cDNA encoding murine B7.1, B7.2, and ICAM-1 and H2-A^d were generated from Con A-stimulated spleen cells by using oligonucleotides based on the published sequences as described (15). These cDNA were sequenced and inserted into the *Drosophila* expression vector pHMRa-3 containing the metallothionein promoter. The constructs were transfected into Schneider SC2 cells as described (15) and stable cell lines selected by culture at room temperature in *Drosophila* medium containing 5% fetal calf serum and 500 μg/ml geneticin (GIBCO/BRL). Selection for cells expressing similar levels of these proteins was achieved by several rounds of cell sorting on a FACStarPlus cell sorter. Forty-eight hours before use, expression of the transfected genes was induced by the addition of $CuS0_4$ at 1 mM.

Assay of Proliferation and Cytokine Production. CD4$^+$ cells were purified from D011 lymph nodes by passage over nylon wool columns followed by antibody and complement-mediated

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Abbreviations: IL, interleukin; IFN-γ, interferon-γ; Th, T helper cell; MHC, major histocompatibility complex; APC, antigen-presenting cell; ICAM, intercellular adhesion molecule-1; TCR, T cell receptor; PE, phycoerythrin; T-S, T depleted spleen APC cell; Ova, ovalbumin; LFA-1, lymphocyte function-associated antigen-1.
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killing of $CD8^+$ T cells, residual B cells, and IA^+ cells as described (18). In some experiments, $CD4^+$ cells were further purified by sorting on a Vantage cell sorter (Becton Dickinson) equipped with high speed sorting capabilities after labeling with fluorescein isothiocyanate-conjugated anti-CD4 antibody and phycoerythrin (PE)-conjugated anti-CD62L or anti-CD44 antibody. A total of $4–5 \times 10^4$ $CD4^+$ cells were cultured at 37°C with 2×10^5 *Drosophila* APC or 5×10^5 mitomycin C-treated, T depleted spleen cells (T-S), and proliferation measured by uptake of [^{3}H]thymidine on days 3–5. Spleen cell suspensions were depleted of T cells by treatment with a mixture of T cell-specific antibodies (J1J, anti-Thy 1.2; 3.168, anti-CD8; and RL172, anti-CD4) and complement. The *Drosophila* cell lines which are propagated at 25°C, die within 24 hr of culture at 37°C, thus excluding any contribution of the *Drosophila* cells to the proliferative response measured. Supernatants were removed from these cultures before the addition of [^{3}H]thymidine for analysis of cytokine production by using a sandwich ELISA assay as described (18). Both capture antibodies and biotinylated detection antibodies were obtained from PharMingen, and recombinant cytokines for construction of standard curves were from Genzyme.

RNase Protection Assay. Assays were performed by using the RiboQuant Multi-Probe RNase Protection Assay System from PharMingen and carried out according to manufacturer's instructions. Briefly, total RNA was purified [by using RNA-STAT60 RNA isolation reagent (TEL-Test "B", Friendswood, TX)] from $CD4^+$ D011 cells cultured for various times with 10 μM ovalbumin (Ova) peptide and the indicated APC. RNA (6.6 μg per sample for 24 hr time point and 10 μg/sample for 48 and 72 hr time points) was hybridized to labeled probes for 13 hr at 56°C and unhybridized probes and RNA digested by incubating with 100 μl of RNase A (192 ng/ml) and RNase T1 (600 units/ml) for 45 min at 37°C. Phenol chloroform extracted probe-target duplexes were separated by electrophoresis on a 5% polyacrylamide sequencing gel and visualized by autoradiography. Radioactivity in the bands was measured by using a Molecular Dynamics PhosphorImager SI. The net cpm per U was calculated as described (19) by using the following formula: [(cpm of cytokine band) − (cpm background around the band)]/no. of U residues in the specific riboprobe. The results are expressed as a percentage of the L32 housekeeping gene.

RESULTS AND DISCUSSION

B7-Expressing *Drosophila* APC Stimulate Strong Primary $CD4^+$ T Cell Proliferative Responses. Transfected cell lines expressing murine MHC class II H2-A^d molecules in combination with B7.1, B7.2, or ICAM-1 were selected by cell sorting. As illustrated in Fig. 1, expression of the transfected molecules is heterogeneous but did not vary markedly between the cell lines used in the experiments described here. To examine APC function, transfected *Drosophila* cell lines were used to present Ova peptide (323–339) to purified $CD4^+$ T cells from the Ova-specific $\alpha\beta$ TCR transgenic line D011 (20). Note that *Drosophila* cells die rapidly at 37°C and do not proliferate when used as APC (15).

In vitro proliferative responses of D011 cells are illustrated in Fig. 1*B*. In the presence of peptide, *Drosophila* APC expressing H2-A^d but lacking additional accessory molecules consistently failed to elicit proliferative responses or cytokine production by naive T cells, which is consistent with the prevailing view that TCR signal transduction alone (signal one) is not sufficient for activation of naive T cells (15, 21). By contrast, H2-A^d-positive *Drosophila* cell lines that coexpressed

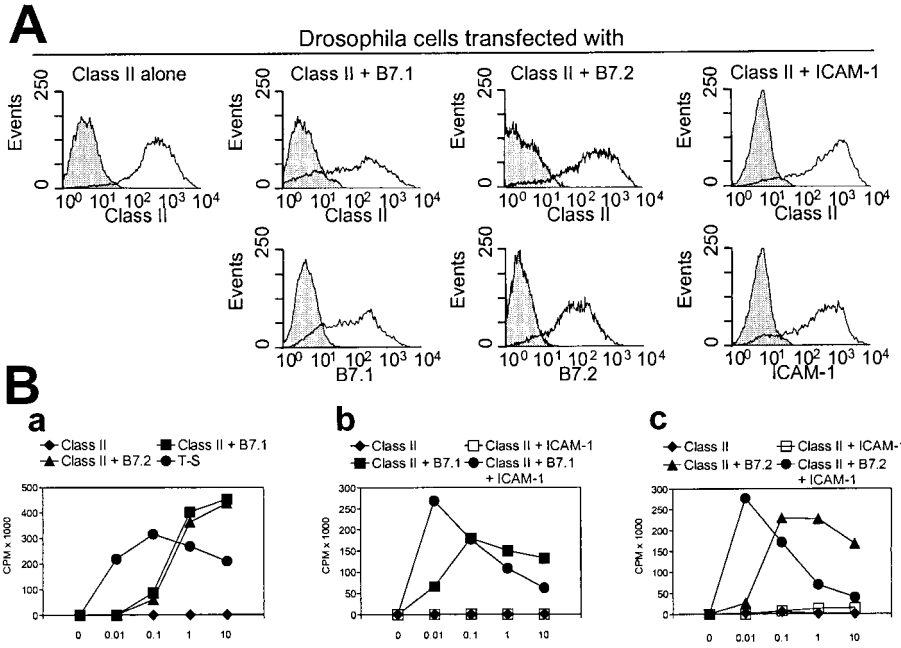

FIG. 1. *Drosophila* cell lines expressing murine MHC class II molecules and defined accessory molecules as APC for $CD4^+$ T cells. *Drosophila* cell lines with stable expression of murine H2-A^d molecules with defined combinations of murine B7.1, B7.2, or ICAM-1 were generated. In *A*, flow cytometry profiles illustrate representative expression of these molecules on four of the cell lines used. Shaded profiles show the nonspecific staining seen with an irrelevant rat antibody (anti-CD4 PE, Becton Dickinson), whereas overlays show the specific staining of the indicated proteins. MKD6 was used to assess class II (H2-A^d) expression, and binding was detected by secondary incubation with rat anti-mouse Ig-PE (Jackson ImmunoResearch). PE-conjugated anti-ICAM-1, anti-B7.1, and anti-B7.2 antibodies were purchased from PharMingen. In *B*, *Drosophila* cell lines expressing the indicated murine molecules were used to stimulate highly purified naïve D011 $CD4^+$ T cells. A total of 5×10^4 $CD4^+$ cells and 2×10^5 *Drosophila* APC were typically cultured in a 300 μl volume with a titration of Ova 323–339 peptide. $CD4^+$ T cells were also cultured with peptide presented by T-S for comparison. Proliferation was evaluated by measurement of [^{3}H]thymidine uptake on days 3–5, and the data show the peak response observed (day 4 for this experiment). Each data point is the mean of triplicate cultures, and the standard error was generally within 20% of the mean.

either B7.1 (class II plus B7.1) or B7.2 (class II plus B7.2) molecules induced strong proliferative responses that were equivalent in magnitude to those seen with T-S (Fig. 1*Ba*). These results confirm the well-documented costimulatory activity of CD28–B7 interactions (16, 22, 23). Compared with splenic APC, however, the class II plus B7 APC were relatively less efficient in that peak responses generally required higher concentrations of Ova peptide.

Augmentation of T cell proliferative responses by interactions involving lymphocyte function-associated antigen-1 (LFA-1) and ICAM-1 is well documented (15, 24–27). In keeping with these findings, coexpression of ICAM-1 and B7 (either B7.1 or B7.2) led to strong proliferative responses with very low concentrations of Ova peptide (Fig. 1 *Bb* and *Bc*). Despite the strong augmentation of proliferative responses conferred by coexpression of ICAM-1 with B7, *Drosophila* APC expressing only ICAM-1 (plus H2-A^d) were very poor stimulators of naïve D011 proliferative responses; thus, even high concentrations of Ova peptide elicited only negligible responses when presented by H2-A^{d+} *Drosophila* cells expressing only ICAM-1 (Fig. 1 *Bb* and *Bc*). Similar findings were seen with purified $CD4^+$ cells from an unrelated TCR transgenic line specific for hemagglutinin peptide (HNT) (data not shown), which makes it unlikely that the D011 transgenic line has unusual requirements for costimulation. These findings contrast with a previous report showing that ICAM-1-transfected mammalian cell lines costimulated $CD4^+$ proliferative responses (27). Although this observation could reflect the coexpression of ICAM-1 and other unknown accessory molecules on the fibroblast cell line studied, ICAM-1 expression on *Drosophila* APC has been found to enhance the response of naive $CD8^+$ cells to MHC class I/peptide complexes (15). Hence there may be fundamental differences in the accessory molecule requirements for cells bearing TCRs with differing affinity for antigen–MHC complexes or in the activation of CD4 vs. $CD8^+$ T cells.

B7 Costimulates Strong IL-4 and IL-10 Production During Primary T Cell Responses. Proliferative responses of naive D011 T cells were in all cases accompanied by IL-2 production, although the amount of IL-2 detected in culture supernatants was variable and no doubt influenced by utilization by the responding T cells (Fig. 2*A*). In general, stimulation with splenic APC led to higher accumulation of IL-2 than stimulation by either class II plus B7.1 or class II plus B7.2 *Drosophila* APC. IL-2 production elicited by *Drosophila* APC tended to be higher when the cells coexpressed B7 and ICAM-1 (data not shown), but even in this situation, IL-2 production was frequently lower than with splenic APC, implying that interactions involving additional accessory molecules may promote production of IL-2.

As for IL-2, splenic APC induced high levels of IFN-γ by D011 $CD4^+$ cells (Fig. 2*B*). In marked contrast, IFN-γ production elicited by *Drosophila* APC was almost undetectable. As discussed below, the failure of *Drosophila* APC to stimulate IFN-γ production reflected, at least in part, active suppression by IL-4 (9–12).

It is generally accepted that IL-4 production is very limited during primary stimulation of naive $CD4^+$ T cells (9–12). Consistent with this view, stimulation of D011 cells with splenic APC led to only minimal accumulation of IL-4 in the cultures, regardless of the concentration of peptide added or the day of assay (Fig. 2*C*). Surprisingly, primary stimulation with *Drosophila* APC expressing either B7.1 or B7.2 (without ICAM-1) elicited extremely high production of IL-4 by naive D011 T cells (Fig. 2*C*); IL-4 production increased linearly with antigen concentration within the range used in these experiments and generally reached maximum levels by day 3–4 of culture. The levels of IL-4 observed were somewhat variable from experiment to experiment but were routinely more than

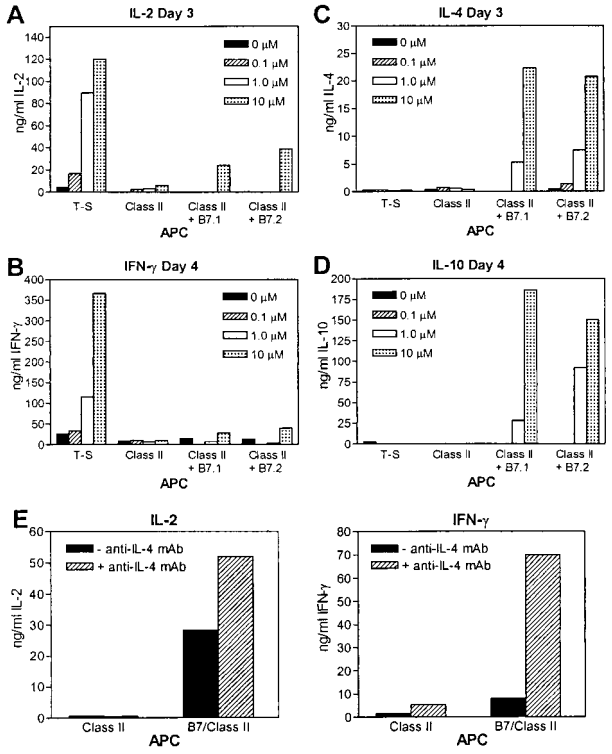

FIG. 2. Cytokines produced after stimulation of D011 $CD4^+$ cells with peptide plus *Drosophila* APC. ELISA assays were used to measure the accumulation of IL-2, IL-4, IL-10, and IFN-γ in the supernatants of cultures set up as in Fig. 1. For *A–D*, supernatants were collected on day 2–4 of assay, and the data shown are the peak responses for the indicated cytokines. The APC were either T-S or *Drosophila* APC expressing the indicated murine molecules. For neutralization of IL-4 (*E*), 11B11 anti-IL-4 antibody (1:2000 dilution of ascites fluid) was added at the initiation of culture, and the indicated cytokines were measured on day 3.

1 log higher than those generally reported in the literature for primary cultures.

It should be emphasized that these data address only the cytokines produced during primary stimulation of D011 $CD4^+$ cells. Secondary responses are associated with distinct patterns of cytokine production; indeed, for D011 $CD4^+$ cells on a BALB/c background, splenic APC induce strong production of IL-4 on secondary restimulation (ref. 28; data not shown). It is also of interest that the production of IL-4 by naive D011 $CD4^+$ cells was similar regardless of whether the *Drosophila* APC expressed B7.1 or B7.2. However, the relevance of these results examining primary responses to previous reports suggesting that ligation of CD28 via B7.1 vs. B7.2 led to the development of T cell subsets secreting different patterns of cytokines (13, 14) is still unclear.

As for IL-4, IL-10 production was virtually undetectable in cultures with splenic APC but very high with *Drosophila* APC expressing B7.1 or B7.2 (Fig. 2*D*). The accumulation of IL-10 showed later kinetics than IL-4 with maximum accumulation occurring on days 4–5 of culture (data not shown).

The observation that *Drosophila* APC elicited very high levels of IL-4 and IL-10 by naive D011 $CD4^+$ cells raised the possibility that one or other of these cytokines suppressed the production of IFN-γ. In this respect other workers have reported that IL-4 is strongly inhibitory for IFN-γ production by Th1 cells (9). In support of this finding, culturing D011 cells with class II plus B7-1 *Drosophila* APC in the presence of anti-IL-4 antibody (at concentrations which effectively neutralized IL-4) increased IFN-γ production by about 10-fold (Fig. 2*E*, *Right*); there was also a smaller (2-fold) increase in IL-2 production (Fig. 2*E*, *Left*). In other studies, IFN-γ

production elicited by class II plus B7 *Drosophila* APC was considerably augmented by addition of IL-12 (data not shown). Likewise, IFN-γ production elicited by splenic APC (a source of IL-12) was reduced by anti-IL-12 mAb; IL-4 production remained unchanged. These data are in line with the view that IL-12 promotes IFN-γ production, probably via a direct action on T cells, and that T cell stimulation by IL-12 counters the inhibitory effect of IL-4 on IFN-γ production.

Though the majority of $CD4^+$ cells from D011 mice express a naive $CD62L^{high}$, $CD44^{low}$, $CD45RB^{high}$ phenotype, it was conceivable that the strong IL-4 and IL-10 production seen after stimulation of D011 $CD4^+$ T cells with peptide presented by class II plus B7 *Drosophila* APC reflected stimulation of a minor subset of activated/memory phenotype cells. To examine this issue, D011 T cells were enriched by passage over nylon wool columns and then sorted for CD4 expression and high or low expression of CD62L, the lymph node homing receptor. As illustrated in Fig. 3, IL-4 production was elicited by both naïve- and memory-phenotype cells. Although the levels of IL-4 were higher with $CD62L^{low}$ memory-phenotype cells, especially at low concentrations of peptide, IL-4 production by naive-phenotype $CD62L^{high}$ D011 cells was clearly substantial. Similar results have been seen in five separate experiments by using cells sorted for CD62L expression as well as in two other experiments by using cells sorted for naive-phenotype $CD44^{low}$ cells (data not shown).

The above data confirm that naive-phenotype $CD4^+$ cells can produce substantial quantities of IL-4 on primary stimulation.

ICAM-1 Down-Regulates Production of Th2-Type Cytokines. Analyses of the cytokines produced after stimulation with class II-expressing *Drosophila* cells coexpressing both B7 and ICAM-1 were surprising. Though IL-2 production was either unchanged or increased, IL-4 production was strongly reduced. Thus, in experiments comparing class II plus B7 *Drosophila* APC and class II plus B7 plus ICAM-1 *Drosophila* APC, only the former induced high levels of IL-4 (Fig. 4). Similar results were seen regardless of the time period studied (2–4 days) and whether B7.1 or B7.2 were used. It should be noted that in multiple experiments involving graded doses of Ova peptide, total recoveries of viable ($TUNEL^-$) or apoptotic ($TUNEL^+$) cells at day 3–7 of culture were similar irrespective of whether B7.2 or B7.2 + ICAM-1-expressing *Drosophila* APC were used. Thus, there was no evidence that the different

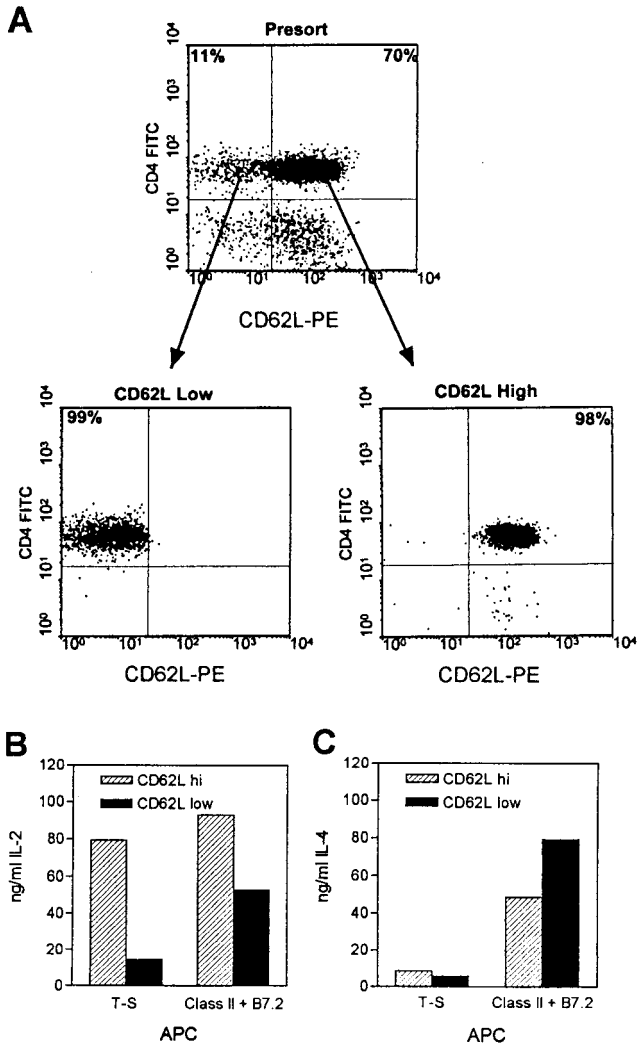

FIG. 3. Cytokine production by $CD4^+$ D011 cells sorted for high or low expression of the lymph node homing receptor CD62L. T cells were enriched from D011 lymph nodes by passage through nylon wool columns, stained with antibody to CD4 and CD62L, and sorted for expression of CD4 and CD62L. (*A*) Dot plots illustrate the presort population (after nylon wool) and the sorted populations after sorting on a VANTAGE flow cytometer. (*B* and *C*) Sorted D011 cells (5×10^4) were cultured with either T-S or *Drosophila* APC expressing class II IA^d and B7.2. IL-2 and IL-4 accumulation was measured on days 2–4 of culture; results from day 3 (peak response in this experiment) are shown.

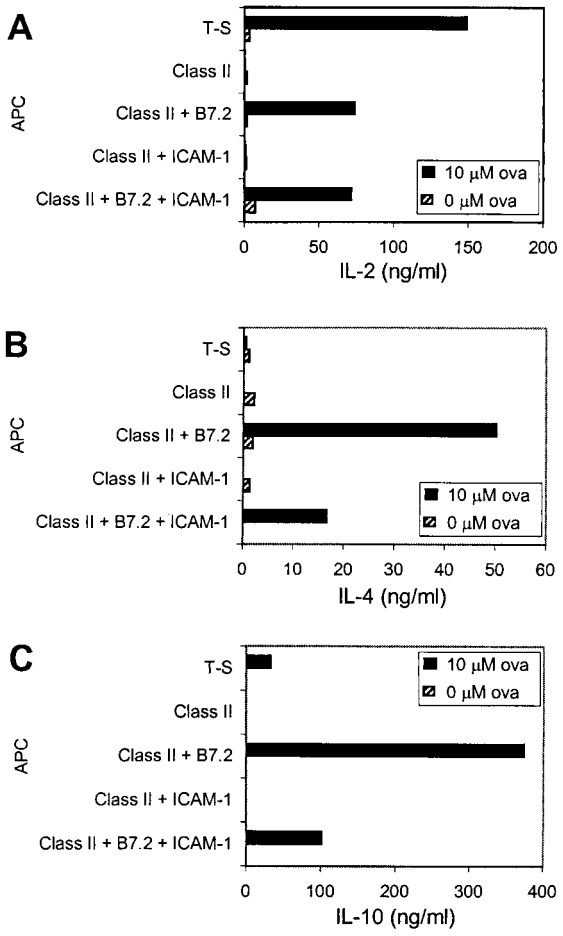

FIG. 4. Coexpression of ICAM-1 and B7 on *Drosophila* APC inhibits IL-4 and IL-10 production. D011 $CD4^+$ T cells were cultured with T-S or *Drosophila* APC expressing the indicated murine accessory molecules. IL-2 (*A*), IL-4 (*B*), and IL-10 (*C*) were measured on days 2–4 of culture, and the peak production was illustrated (day 3, IL-2; day 4, IL-4, IL-10). Ova peptide was titrated as in Fig. 2; as maximum production of these cytokines was seen with 10 μM Ova, only data from cultures with this concentration are shown.

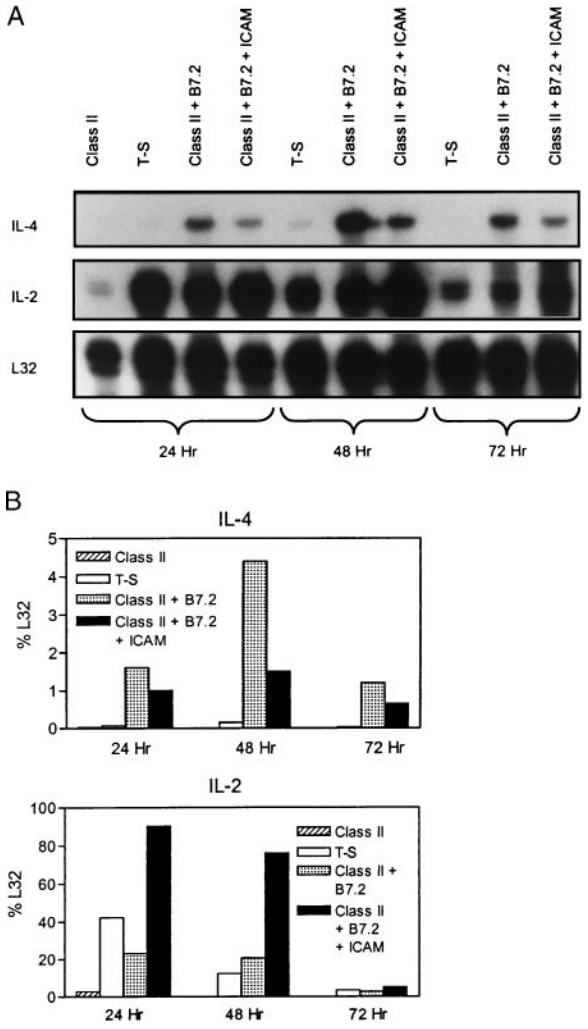

FIG. 5. Reduced production of IL-4 after stimulation with ICAM-1-expressing *Drosophila* APC is apparent at the level of mRNA. D011 cells were cultured with 10 μM Ova peptide presented by either T-S or *Drosophila* APC expressing the indicated accessory molecules. RNA purified after 24, 48, and 72 hr of culture was assessed for cytokine gene expression by using a multiprobe RNase protection assay (as described). (*A*) A representative autoradiograph for IL-4, IL-2, and the housekeeping gene L32, is shown. (*B*) The radioactivity in the bands was quantitated by using a PhosphorImager and the results plotted as percentage of L32.

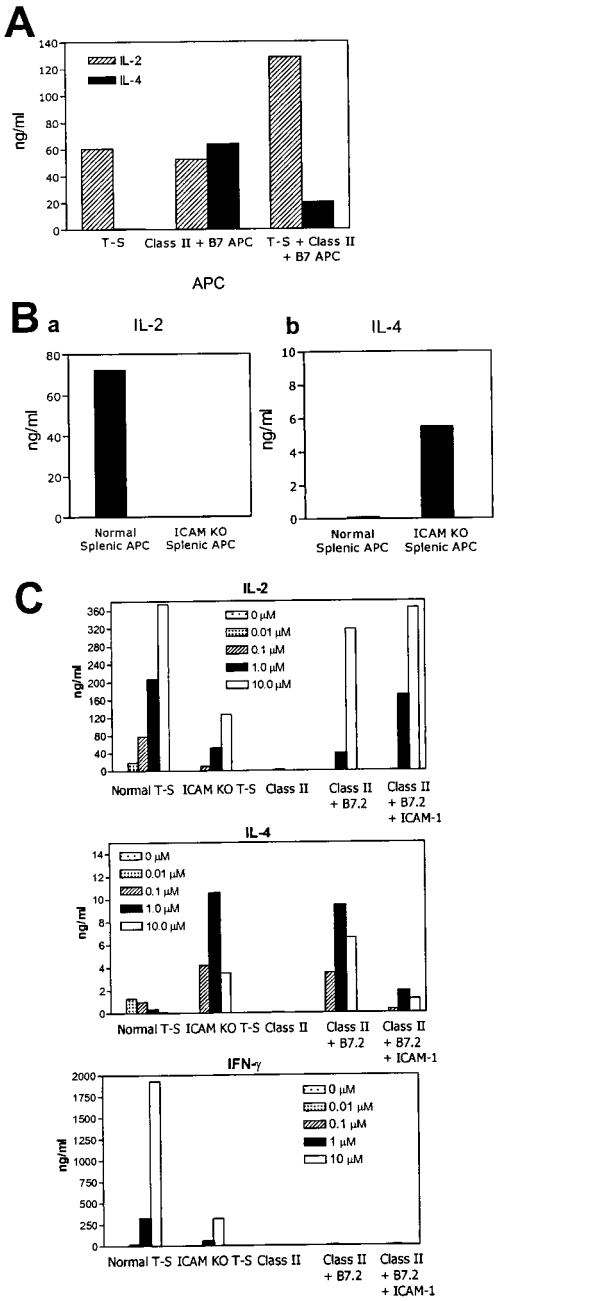

FIG. 6. ICAM-1 expression by splenic APC down-regulates IL-4 production. (*A*) D011 $CD4^+$ T cells were cultured with limiting numbers of T-S alone (2.5×10^5), with *Drosophila* APC expressing class II H2-A^d plus B7–1 (1.25×10^5), or with a mixture of T-S (2.5×10^5) and *Drosophila* APC (1.25×10^5). IL-2 and IL-4 production was measured on day 4. (*B*) $CD4^+$ T cells purified as in Fig. 1 from normal D011 mice were cultured with Ova peptide and T-S from normal mice or ICAM-1-deficient mice. The indicated cytokines were measured on supernatants taken at various times after culture with 0.01–10 μM Ova; peak responses at day 3 with 10 μM Ova are shown. The peak proliferative response to Ova was equivalent with normal and ICAM-1-deficient APC, though responses with ICAM-1 deficient APC required higher concentrations of peptide. (*C*) Nylon wool purified D011 lymph node cells were FACS sorted for CD4 expression and high expression of CD62L. These naive phenotype cells were cultured with a titration of Ova peptide and with splenic APC (either from normal or ICAM-1-deficient mice) or with *Drosophila* APC expressing the indicated accessory molecules. Cytokines were measured on days 1–4 and the peak responses are shown: for IL-2, day 3, for IL-4 and IFN-γ, day 4.

levels of IL-4 induced by B7.2 vs. B7.2 plus ICAM-1 APC reflected the degree of T cell expansion/viability.

To exclude the possibility that the difference in IL-4 measured in these cultures reflected differential usage of this cytokine by the responder T cells, we examined the level of mRNA by using a multiprobe RNase protection assay. As shown in Fig. 5, IL-4 mRNA was detectable as early as 24 hr poststimulation with B7.2-expressing *Drosophila* cells, whereas levels were substantially less when mRNA was purified from $CD4^+$ cells stimulated with either T-S or B7.2 plus ICAM-1 *Drosophila* APC. This difference in mRNA levels was even more accentuated at 48 hr poststimulation where the levels of IL-4 mRNA reached a maximum in cells stimulated with ICAM-1 negative APC.

The above findings on IL-4 protein and mRNA raised the possibility that ICAM-1, which is strongly expressed by normal splenic APC, could be involved in actively inhibiting IL-4 production in primary T cell responses. Consistent with this possibility, stimulation of D011 $CD4^+$ cells with a mixture of ICAM-1-expressing normal splenic APC and class II plus B7 *Drosophila* APC was associated with much reduced production of IL-4 (Fig. 6*A*) and IL-10 (data not shown).

To test directly the notion that ICAM-1 normally plays a role in down-regulating IL-4 production by naive T cells, we examined the production of IL-4 in cultures utilizing splenic APC from ICAM-1-deficient mice (see *Materials and Methods*) and either unsorted D011 $CD4^+$ cells (Fig. 6*B*) or fluorescence-activated cell sorter (FACS)-sorted naive ($CD62L^{high}$) D011 $CD4^+$ cells (Fig. 6*C*). Whereas stimulation of D011 $CD4^+$ cells with normal splenic APC failed to induce substantial IL-4 production, ICAM-1-deficient splenic APC stimulated significant production of both IL-4 (Fig. 6 *B* and *C*) and IL-10 (data not shown). By contrast, IL-2 and IFN-γ production was lower but still significant with ICAM-1-deficient splenic APC (Fig. 6 *B* and *C*); adding anti-IL-4 mAb to the cultures failed to markedly enhance IFN-γ production (data not shown), implying that limited IFN-γ production elicited by ICAM-1^- APC did not simply reflect inhibition by IL-4.

How ICAM-1 inhibits IL-4 production is not yet known. A role for "strength" of T cell signaling in determining the balance of Th1 and Th2 cells in immune responses has been previously suggested based on studies examining the effects of antigen concentration, altered peptide ligands, and intensity of CD28 costimulation on cytokine production (16, 29, 30). Because ICAM-1 binding to LFA-1 on T cells increases adhesion and thus may promote TCR-mediated signal transduction, our results are consistent with the notion that strong TCR signaling is inhibitory for IL-4 production by naïve T cells.

Another possibility that is not mutually exclusive is that ICAM-1 ligation of LFA-1 on naïve T cells (or another T cell accessory receptor) delivers signals that directly interfere with the induction of IL-4 mRNA. This possibility would be in line with the observation that ICAM-1 expression on *Drosophila* APC down-regulated IL-4 production even when very low doses of peptide were used for stimulation.

Regardless of the mechanisms involved, the present data suggest that ICAM-1-mediated down-regulation of IL-4 production plays an important role in regulating the cytokines produced by $CD4^+$ cells in primary T cell responses. Thus, the key finding reported here is that, in the absence of ICAM-1, naive T cells are capable of producing high levels of both IL-4 and IL-10—i.e., cytokines that are generally thought to be secreted primarily by previously activated T cells. The failure to detect significant levels of IL-4 and IL-10 in typical primary responses may thus simply reflect that the APCs controlling stimulation of naive T cells express high levels of ICAM-1.

Note Added in Proof. Since the submission of this manuscript, other workers have reported that antibody blocking of LFA-1-ICAM interactions in cultures promotes Th2 cytokine production (31).

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