Cutting Edge: LFA-1 Interaction with ICAM-1 and ICAM-2 Regulates Th2 Cytokine Production

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The role of CD28/B7 and LFA-1/ICAM costimulation in proliferation and Th1/Th2 differentiation of naïve CD4+ T cells was addressed using T cells from DO11.10 TCR transgenic mice stimulated by dendritic cells. The blockade of either CD28/B7 or LFA-1/ICAM interactions partially inhibited T cell proliferation. By comparison, blocking CD28/B7 costimulation inhibited IL-4 and IL-5 (Th2 cytokine) production, whereas blocking LFA-1/ICAM-1 or LFA-1/ICAM-2 led to a significant increase (15- to 40-fold) of Th2 cytokines. The combination of anti-ICAM-1 and anti-ICAM-2 mAbs had a synergistic effect with a 100- to 1000-fold increase of Th2 cytokine production. Thus, these two costimulatory pathways have opposing roles in the regulation of Th2 development.

In this study, we examined the role of CD28/B7 and LFA-1/ICAM interactions in the regulation of T cell proliferation and differentiation of naïve T cells from DO11.10 transgenic mice that express a TCR specific to OVA 323–339 (13). The data suggest that the CD28/B7 and LFA-1/ICAM pathways have opposing roles in the regulation of naïve T cell differentiation stimulated by “fresh” CD8+ DCs, the most potent immunostimulatory cells of the spleen (9). Thus, DCs may promote Th1 development by suppressing Th2 development through the interaction of ICAM-1 and ICAM-2 with LFA-1.

Materials and Methods

Mice

BALB/c mice were purchased from Frederick Cancer Research Facility (Frederick, MD) and maintained in a specific pathogen-free barrier facility at the University of Chicago (Chicago, IL). DO11.10 transgenic mice expressing a TCR specific for OVA peptide 323–339 presented in the context of I-Aa(13) were bred to BALB/c mice and then to Rag-2-deficient-mice (gift from Fred Alt, Harvard Medical School, Boston, MA) to generate DO11.10 × Rag-2−/− mice. TCR transgene expression and B cell deficiency were analyzed by FACS.

Culture medium, Abs, blocking reagents

mAbs 145-2C11 (anti-CD3 (14)), J11d (anti-murine CD24, ATCC TIB-183; American Type Culture Collection (ATCC), Manassas, VA), and MKD6 (anti-murine I-Aa(15)) were prepared in our laboratory. Purified mAbs Y1/1.7.4 (anti-ICAM-1, ATCC CRL-1878) and M17/4.2 (anti-ICAM-2, ATCC TIB-127) were a gift from Dr. Robert Hendricks (University of Pittsburgh, PA), and ascites of Y1/1.7.4 were a gift from Dr. Jim Miller (University of Chicago). M2C24 (anti-ICAM-2) mAb was purchased from PharMingen (San Diego, CA). The murine (m)CTLA4Ig was obtained from Genetics Institute (Cambridge, MA).

Purification of T cells and APCs

T cells from spleen and lymph nodes of DO11.10 × Rag-2−/− mice were purified after passage over nylon wool columns, depletion of cells expressing CD24 (J11d) and MHC class II (MKD6) with rabbit complement at 37°C for 45 min, and Ficol-Hypaque gradient separation. For DC purification, low density BALB/c spleen cells were prepared as previously described (16, 17). The recovered low density cells, preincubated with 2.4G2 mAb to reduce nonspecific binding, were stained with biotin-labeled N418 from Genetics Institute (Cambridge, MA).

Proliferation assays

Six thousand T cells were cultured with 2,000 irradiated CD8+ DCs or 20,000 irradiated splenocytes depleted of DCs (1000 rad) in round-bottom
96-well culture plates in DMEM (Life Technologies, Grand Island, NY). The medium was supplemented with 10% FCS (Summit Biotechnology), 100 U/ml penicillin, 100 μg/ml streptomycin, 0.29 mM L-glutamine, nonessential amino acids, 10 mM HEPES, 5 × 10⁻⁵ M 2-ME, and 1 μg/ml OVA 323–339 peptide (University of Chicago Peptide Synthesis Facility). Proliferative responses were assessed at 96 h by adding 1 μCi/well of [³H]thymidine during the final 7–9 h of culture before harvesting and scintillation counting.

T cell stimulation for cytokine production

T cells (10⁴/well) were stimulated in the same culture medium used for the proliferation assay with 1 μg/ml OVA peptide and purified CD8⁻ DCs (2 × 10⁴/well) in round-bottom 96-well culture plates. In some assays, supernatants (20 μl/well) were collected at 40 h and analyzed for IL-2 by ELISA. Alternatively, after 5 or 7 days, cells for each culture condition were pooled, and live cells were recovered by Ficol-Hypaque gradient centrifugation, washed twice in PBS, and replated (2 × 10⁶ cells/well in 400 μl) onto anti-CD3-coated (145-2C11 at 1 μg/ml) 48-well plates. Supernatants were harvested after 44 h and analyzed for cytokines by ELISA (IL-2, IL-4, and IL-5 using commercial kits from Endogen, Cambridge, MA, and IFN-γ using reagents kindly provided by Dr. Robert Schreiber, Washington University (St. Louis, MO).

Results and Discussion

CD8⁻ DCs are the major splenic APC for naive DO11.10 T cells

In the spleen, the CD8⁻ DC subpopulation is considered the critical initiator of primary immune responses compared with the other DC subset and other APCs (9, 17). In this study, Ag-specific T cells from DO11.10 × RAG–2⁻/⁻ transgenic mice were used in in vitro assays to compare DCs and other APCs in naive T cell proliferation and differentiation. DC subpopulations and other APCs of the spleen were purified by multiparameter flow cytometric cell sorting to avoid the necessity for in vitro culture, which could modify their physiologic properties. Naive CD4⁺ T cells were very poorly stimulated by DC-depleted splenocytes. In contrast, proliferation of the TCR transgenic cells stimulated by 10-fold less CD8⁻ DCs was increased by 50-fold. (Fig. 1A). These results indicate that DCs are the major, if not the only, APCs able to stimulate naive DO11.10 T cells in vitro. Thus, additional experiments utilized the CD8⁻ DC population to study the role of costimulation in proliferation and differentiation of naive T cells.

CD4⁺ T cell proliferation in the presence of reagents that block either CD28/B7 or LFA-1/ICAM interactions

The role of B7 in the proliferation of naive CD4⁺ T cells was assessed by stimulating naive DO11.10 T cells with syngeneic DCs in the presence of 1 μg/ml OVA peptide and 5 μg/ml mCTLA4Ig (a soluble CD28 antagonist that binds with high affinity to the B7-1 and B7-2 molecules). IL-2 production at day 2 was inhibited by 80% by mCTLA4Ig, confirming the role of B7 costimulation in IL-2 production (18). In contrast, T cell proliferation was inhibited by only ~50% at day 4 with mCTLA4Ig (Fig. 1B). This result differs from previous reports in which late proliferation of T cells stimulated by total splenocytes was almost completely inhibited in the presence of CD28 blockade (19, 20). However, in those systems, macrophages and B cells may play a role in the stimulation of T cells, especially as mixed naive and memory T cell populations may be present in these RAG⁺ mice. In fact, contrary to naive T cells, activated T cells can be efficiently stimulated by B cells (21). Thus, stimulation of pure naive T cells by DCs, as opposed to mixed T cell populations stimulated by the different splenic APCs, may be less dependent on CD28 signaling for proliferative responses. One explanation for the inability of mCTLA4Ig to fully block T cell proliferation is that DCs express other costimulatory molecules that stimulate the T cells.

Blocking mAbs specific to ICAM-1 and ICAM-2 were used for the examination of the role of LFA-1/ICAM interactions in Ag-specific T cell proliferation in this model. The addition of blocking anti-ICAM-1 mAb had only a minimal effect on both proliferation and IL-2 growth factor production of DO11.10 T cells stimulated by DCs as compared with control cultures. Furthermore, blocking ICAM-2/LFA-1 interaction did not inhibit T cell activation. There were no additive effects of blocking both ICAM-1 and ICAM-2 (Fig. 1B). Conflicting results have been reported on the role of ICAM/LFA-1 in T cell proliferation. In artificial systems of T cell stimulation by insect cells or fibroblasts transfected with MHC and costimulatory molecules or by immobilized ICAM-1 and mAb to CD3, ICAM-1/LFA-1 seems to be a major costimulatory pathway (22–24). Other studies using blocking mAbs or ICAM-1- and LFA-1-deficient mice have concluded that ICAM-1/LFA-1 interactions are dispensable in many settings (25–28). Our results suggest that proliferation of naive DO11.10 CD4⁺ T cell stimulated
As stated above, costimulatory events have been shown to effect the differentiation of Th cells both in vitro and in vivo. Thus, the role of CD28/B7 and LFA-1/ICAM-1 costimulation in Th differentiation was examined. Naïve CD4+ T cells from DO11.10 × RAG2−/− were cultured with DCs and 1 μg/ml (0.5 μM) OVA 323–339 for 5 days. Since levels of IFN-γ, IL-4, and IL-5 cytokines were not detected in the primary culture (data not shown), cells were harvested and restimulated with immobilized anti-CD3. IL-4, IL-5, and IFN-γ secretion was measured after 44 h. A representative experiment from two independent experiments is shown.

FIGURE 2. Opposite effects of blocking B7 and ICAM-1 costimulation on Th2 cytokine production. DO11.10 T cells (10^6/well) were cultured with CD8+ spleen DCs (2 × 10^6/well), OVA peptide, and no blocking reagent (media) or either blocking CD28/B7 (mCTLA4Ig at 5 μg/ml) or LFA-1/ICAM-1 (anti-ICAM-1, 10% ascites of Yn1 mAb) interactions. After 5 days of primary stimulation, blast T cells were harvested and restimulated with immobilized anti-CD3. IL-4, IL-5, and IFN-γ secretion was measured after 44 h. A representative experiment from two independent experiments is shown.
In summary, our findings show that the LFA-1/ICAM interactions have a major role in the regulation of IL-4 and IL-5 production by CD4⁺ T cells. The effect appears to be dominant because the ligation of CD28/B7 alone promotes Th2 cytokines, while co-ligation of LFA-1/ICAM is inhibitory. It should be emphasized that the effect of LFA-1/ICAM blockade is not limited to the DO11.10 system described herein. In separate studies, we have observed that the Th2 cytokines produced by C57BL/6 T cells responding to BALB/c APCs in an allogeneic MLR are enhanced by the addition of either anti-LFA-1 or anti-ICAM-1 mAbs (data not shown). Thus, the regulation of Th1/Th2 balance by LFA-1/ICAMs is effected in at least three different systems: Ag-specific stimulation; MLR and anti-CD3 Ab stimulation. In addition, Th2 cytokine production was observed using T cells from the Th2-prone BALB/c mice, the Th1-prone C57BL/6 mice, and human T cells (33).

The mechanism by which LFA-1/ICAM interactions suppress Th2 development is unclear. However, it is unlikely that decreased adhesion or the strength of signal 1 is responsible. We believe it is more likely that unique signals are delivered via this pathway, either into the T cells or perhaps the DCs that inhibits Th2 development. In a simple system in human, coimmobilized anti-CD3 and ICAM-1 resulted in the differentiation of naive T cells into IFN-γ-secreting cells consistent with the existence of LFA-1 signaling into T cells (33). Our results are particularly relevant to ongoing clinical studies in which the benefits of blocking LFA-1/ICAM interactions in allograft rejection and autoimmunity are being examined. Our findings suggest that part of the beneficial effects of these therapies may be mediated by an increase of Th2 cytokines, which have anti-inflammatory properties (1).

Note added in proof. Similar observations that Th2 cytokine production is regulated by ICAM/LFA-1 interaction has been reported by C. R. Luksch, O. Winqvist, N. E. Ozaki, L. Karlsson, M. R. Jackson, P. H. Peterson, and S. R. Webb.

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References


