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ICAM-1 costimulation induces IL-2 but inhibits IL-10 production in superantigen-activated human CD4$^+$ T cells

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SUMMARY

We have previously reported that costimulatory pathways including B7–CD28 and lymphocyte function-associated antigen-3 (LFA-3)–CD2 shape distinct activation profiles in human CD4$^+$ T cells. We now show that superantigen (SAg), in combination with intracellular adhesion molecule-1 (ICAM-1) costimulation drives a proliferative response accompanied by high levels of interleukin-2 (IL-2) and moderate levels of interferon-γ (IFN-γ) and tumour necrosis factor (TNF). This response profile differs from that observed in B7 or LFA-3 costimulated T cells because our previous results showed that B7–CD28 costimulation was accompanied by high levels of IL-2, IFN-γ and TNF, whereas LFA-3 was a potent inducer of IFN-γ and TNF, but had little influence on IL-2 production. The ICAM-1-induced IL-2 production could efficiently be abrogated with monoclonal antibody (mAb) against ICAM-1 or LFA-1, showing that the activation is dependent of a functional ICAM-1–LFA-1 pathway. SAg-induced IL-2, IFN-γ and TNF were detected in both CD4$^+$ and CD8$^+$ T cells, whereas production of IL-10 was restricted to CD4$^+$ T cells. A major finding in the present study was that ICAM-1 costimulation strongly inhibits IL-10 production in CD4$^+$ T cells. Our data demonstrate that ICAM-1 costimulation is sufficient to induce large amounts of IL-2. The presence of ICAM-1 results in suppression of IL-10 production in T helper (Th) cells, which may favour the development of Th1 and not Th2 T cells.

INTRODUCTION

At least two signals are required for full activation of T lymphocytes. The first, antigen-dependent signal is generated by the engagement of the T-cell receptor (TCR)/CD3 complex on the T cell with the major histocompatibility complex (MHC)/peptide complex on an antigen-presenting cell (APC). A second, costimulatory signal is provided by several distinct molecules present on the surface of the APC,[1] including intracellular adhesion molecule-1 (ICAM-1), lymphocyte function-associated antigen-3 (LFA-3) and B7 and the corresponding LFA-1, CD2 and CD28 receptors, respectively, on the T cell.[2–4] It has been shown that these molecules both provide an initial adhesive contact between the APC and the T cell, and may contribute to T-cell activation by transducing costimulatory signals.[5–7] Absence of a costimulatory signal can induce a state of unresponsiveness or clonal anergy.[1,8,9]

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Abbreviations: LFA-1, leucocyte function-associated antigen; ICAM, intercellular adhesion molecule; TNF, tumour necrosis factor; IFN-γ, interferon-γ; PBL, peripheral blood lymphocytes; SEA, staphylococcal enterotoxin A.

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We have used the bacterial superantigen (SAg) staphylococcal enterotoxin A (SEA) presented on target cells transfected with MHC class II and various costimulatory ligands in order to mimic, in a genetically defined manner, the two-signal system of T-cell activation. Using this system, we have previously shown that the costimulatory molecules B7, and LFA-3 induce distinct activation profiles in SAg-activated T cells. Costimulation with B7 resulted in a vigorous response with production of high levels of interleukin-2 (IL-2), interferon-γ (IFN-γ) and prolonged proliferation, whereas transient proliferation and low levels of IL-2 were seen after LFA-3 costimulation.[10] Although B7 has been shown to be a strong inducer of IL-2 production and induction of proliferation and effector functions in CD4$^+$ as well as CD8$^+$ T cells[11,12] several studies have shown a co-operative effect of B7 and the ICAM-1 in T-cell costimulation to proliferation and effector functions including antitumour T-cell immunity.[13,14] Furthermore, recent studies using allogeneic Epstein–Barr virus (EBV)-transformed B cells as SAg-presenting cells have suggested that the adhesive interaction between LFA-1 on T cells and its counter-receptor on SAg-presenting cells is necessary to support SAg-induced proliferation of T cells.[15] However, only limited information is available on the effect of ICAM-1 costimulation on the T-cell cytokine profile. Therefore, in the present study we have evaluated whether costimulation with ICAM-1 results in a unique cytokine profile in T cells.

The ICAM family of adhesion molecules is distinctly expressed in various tissues, ICAM-1 can be induced on a variety of cells in response to IL-1 and IFN-γ.[4] ICAM-2 is expressed on resting endothelial cells and most leucocytes while ICAM-3 primarily is expressed on resting leucocytes. These observations suggest, a possible role for ICAM-2 in normal recirculation of resting T cells,[16] participation of ICAM-3 in the initiation of a immune response[17] and indicate that ICAM-1 might be involved later in an inflammatory response.[4,18,19]

We demonstrate a proliferative response in both $CD4^+$ and $CD8^+$ T cells exposed to SEA on L cells cotransfected with ICAM-1 and DR7 Moreover, ICAM-1 costimulation resulted in production of substantial amounts of IL-2 and moderate levels of IFN-γ and tumour necrosis factor (TNF). Most interestingly, $CD4^+$ T cells, exposed to LDR7 cells lacking ICAM-1, produced significant amounts of IL-10, whereas only marginal levels of IL-10 were seen after ICAM-1 costimulation.

IL-10 is a pleiotropic cytokine produced by T helper (Th)0 and Th2 helper T cells, as well as by B cells and monocytes in the mouse. IL-10 has thus been suggested to suppress cytokine synthesis and proliferation of Th1 cells.[20–22] Several studies have implicated that IL-10 may serve as a physiological feedback mechanism to down-regulate IFN-γ production induced during the early immune response.[20,23,24]

Our results indicate that ICAM-1 costimulation inhibits IL-10 production from human SEA-stimulated $CD4^+$ T cells, thereby most likely favouring a pro-inflammatory Th1-driven immune response.

MATERIALS AND METHODS

Reagents

The monoclonal antibodies (mAb) CD44–fluoroscein isothiocyanate (FITC), CD71–FITC, CD25–phycoerythrin (PE), CD11a–FITC, human leucocyte antigen (HLA)-DR–PE, CD80–PE (B7.1) and FITC–goat $F(ab)_2$ antimouse were purchased from Becton Dickinson Laboratories, San Jose, CA. The mAb CD40L–PE, IL-2Rβ–PE, anti-B70–PE (B7.2), PE-conjugated streptavidin, rat antihuman IL-10, biotinylated rat antihuman IL-10 and recombinant human interleukin 10 (IL-10) were purchased from Pharmingen, San Diego, CA. FITC–rabbit $F(ab)_2$ antimouse immunoglobulins were purchased from Dakopatts, Glostrup, Denmark. Rat antihuman Tugh4 (IL-2Rγ) was a kind gift from Dr K. Sugamura, Tohoku University School of Medicine, Tohoku, Japan and mouse antirat Fab immunoglobulin–FITC was purchased from Southern Biotech Associate Inc., Birmingham, U K. Anti-ICAM-1 mAb was generously provided by Dr A. Boyd, Walter and Elisa Hall Institute, Melbourne, Australia.[25] Ficoll–Paque and Percoll were purchased from Pharmacia Inc., Uppsala, Sweden. Peroxidase Substrate Kit for enzyme-linked immunosorbent assay (ELISA) were purchased from Bio-Rad Laboratories, Hercules, CA. Recombinant SEA was expressed in *Escherichia coli* and purified to homogeneity as described.[26]

Media used

Complete medium: RPMI-1640 medium (Gibco, Paisley, UK) supplemented with 10 mM HEPES, 4 mM L-Glutamine, 1 mM pyruvate, 0·1% $NaHCO_3$ and 10% fetal calf serum; phosphate-buffered saline (PBS) (0·68 mM Ca^{2+}, 0·49 mM Mg^{2+}).

Transfected cell lines

L-cell transfectants expressing HLA-DR7 (LDR7) or HLA-DR7 and ICAM-1 (LDR7/ICAM-1) gene products have been described[18,27] The cell lines were kept in appropriate hypoxantine/mothotrexate/thymidine (HMT) and G418 selection media.[27]

Cell separation and culture

Human mononuclear cells were isolated from buffy coats by centrifugation on Ficoll–Paque and Percoll gradients and depleted of monocytes by the use of a gelatin column.[28] $CD3^+$ T cells were purified with a negative panning selection technique using mAbs, as described earlier.[27,29] $CD4^+$ and $CD8^+$ T-cell subsets were obtained by using the magnetic cell sorter (MACS) cell sorting system (Miltenyi Biotec, Sunnyvale, CA) according to the manufacturer's recommendations. The purity of the T cells regularly exceeded 98%, as determined by fluorescence-activated cell sorting (FACS) analysis.

Immunofluorescence analysis

Two-colour staining was performed using standard settings on a FACScan flow cytometer (Becton Dickinson).

T-cell stimulation

Freshly isolated human T cells (2×10^5 cells/well), were cultured in 96-well, flat bottom microtitre plates with mitomycin C-treated (0·1 mg/ml for 1 hr) L transfectants (2×10^4 cells/well) in the presence or absence of SEA (0·6 ng/ml). Supernatants were collected after 24 hr, 3 days, 5 days, 7 days or 10 days of incubation and analysed for IL-2, IL-10, IFN-γ and TNF content. The cells were then pulse labelled with [^{3}H]thymidine for 6 hr and harvested onto a filter paper or analysed for expression of the cell surface molecules IL-2Rα, IL-2Rβ, IL-2Rγ, B7.1 (CD80), B7.2 (CD86), CD40L, CD69, CD44, CD71 and CD11a after 24 hr using a FACScan flow cytometer (Becton Dickinson). The thymidine incorporation was measured in a β-scintillation counter. For mAb blocking experiments the mitomycin C treated L cell transfectants were preincubated for 30 min with anti-ICAM-1 mAb (1:1000), anti-LFA-1 (IB4, 2·5 μg/ml) or immunoglobulin control mAb (C215, 5 μg/ml).

Cytokine assays

The IFN-γ activity in culture supernatants was assessed in an antiviral cytopathic assay using WISH cells and 3-[4,5 dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) as previously described.[30] The TNF activity in the culture supernatants was assayed using the TNF sensitive WEHI 164 clone 13 cell line as described.[31] IL-2 activity in the culture supernatants was assessed utilizing the IL-2-dependent murine T-cell line CTLL-2 as described.[30] The amount of IL-10 in the culture supernatants was measured with ELISA (Pharmingen) according to the manufacturer's recommendations.

RESULTS

ICAM-1 costimulates proliferation in SEA-triggered CD4$^+$ and CD8$^+$ T cells

We have studied the ability of ICAM-1 to induce a costimulatory signal in SEA-activated human T-cell subsets. Both CD4$^+$ and CD8$^+$ T cells stimulated with SEA in the presence of L cells cotransfected with DR7 and ICAM-1 showed a marked proliferation after 3 days of stimulation. In contrast, no or minimal proliferation was seen after stimulation with untransfected L cells, L cells transfected with DR7 alone (Fig. 1) or L cells transfected with ICAM-1 alone (data not shown) at this time point. The ICAM-1-induced proliferation was increased at day 5 of stimulation and remained high in both T-cell subsets at day 7 (Fig. 1a). Moreover, the proliferative response differed among CD4$^+$ and CD8$^+$ T cells. In the CD4$^+$ T-cell population the response was only partly dependent on ICAM-1, whereas the CD8$^+$ cells required ICAM-1 costimulation to respond.

ICAM-1 induces IL-2 but inhibits IL-10 production from SEA stimulated CD4$^+$ T cells

The proliferative response seen after ICAM-1 costimulation of SEA-activated CD4$^+$ or CD8$^+$ T cells was accompanied by elevated levels of IL-2 (Fig. 2a,b). In contrast, CD4$^+$ T cells stimulated with the single HLA-DR transfected L cells produced very little IL-2 but significant levels of IL-10 (Fig. 2c). No IL-10 was produced by the CD8$^+$ T cells, regardless of stimulation (Fig. 2d). Interestingly, low or no IL-10 production was seen after ICAM-1 costimulation of CD4$^+$ T cells (Fig. 2c), suggesting that costimulation with ICAM-1 inhibits IL-10 production. Moreover, elevated levels of IFN-γ and TNF could be detected in supernatants from both CD4$^+$ and CD8$^+$ T cells costimulated with ICAM-1 (Fig. 2e–h).

In order to further evaluate the importance of the IL-10 versus IL-2 production we performed a kinetics analysis. Maximal IL-2 production in CD4$^+$ T cells was recorded after 3 days of ICAM-1 costimulation (Fig. 3a). However, a marginal IL-10 production was seen in CD4$^+$ T cells costimulated with ICAM-1 at this time point (Fig. 3b), presenting an IL-10/IL-2 relative ratio of $<0{\cdot}5$ after 3 days of stimulation (Table 1). In contrast, lack of ICAM-1 stimulation resulted in an IL-10/IL-2 ratio of >10 (Table 1). Moreover, after 7 days of stimulation there was a major increase in IL-10 production from the LDR7 stimulated cultures, whereas no IL-2 was detected at this time point. Although the IL-10/IL-2 ratio from ICAM-1 costimulated T cells increased considerably at the later time points studied it remained at least 20-fold

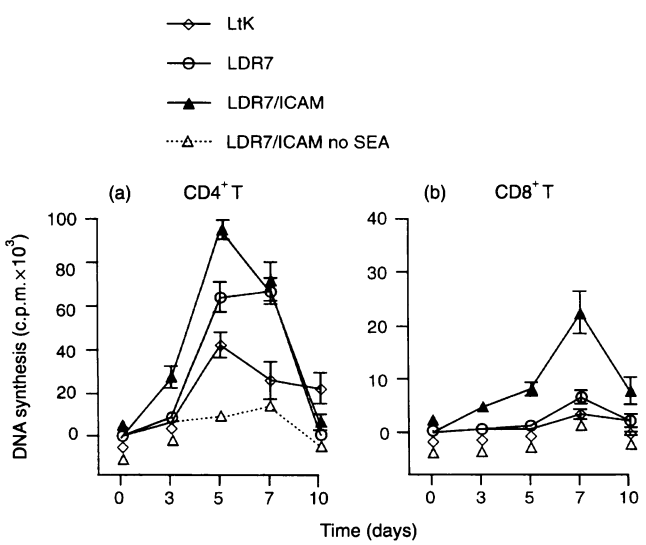

Figure 1. ICAM-1 costimulates CD4$^+$ and CD8$^+$ T cells. Kinetics of proliferation of CD4$^+$ (a) or CD8$^+$ T cells (b) in the presence of SEA (0·6 ng/ml) and L cell transfectants as indicated above. Ltk represents untransfected L-cells. One representative experiment out of four. Mean value ± SEM is shown for triplicate cultures.

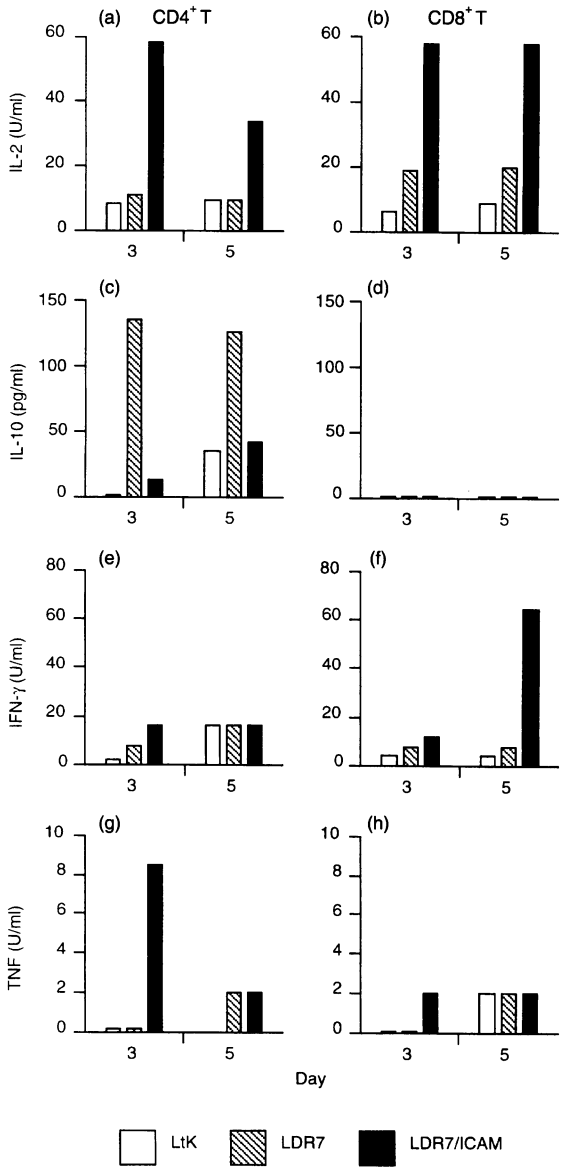

Figure 2. ICAM-1 costimulates cytokine production in CD4$^+$ and CD8$^+$ T cells. IL-2 (a and b), IL-10 (c and d), IFN-γ (e and f) and TNF (g and h) production following 3 or 5 days of costimulation in CD4$^+$ or CD8$^+$ T cells in the presence of SEA (0·6 ng/ml). One representative experiment out of three. Mean value ± SEM of triplicates is shown.

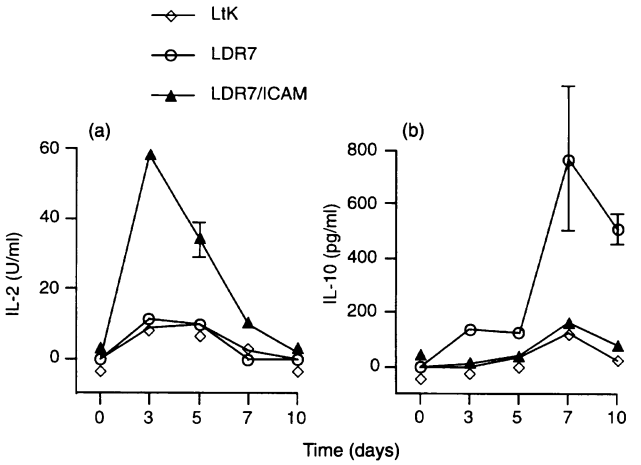

Figure 3. ICAM-1 inhibits IL-10 production from SAg-triggered $CD4^+$ T cells. Kinetics of IL-2 (a) or IL-10 (b) production from $CD4^+$ T cells costimulated in the presence of SEA (0·6 ng/ml) and L cell transfectants as indicated. One representative experiment out of four. The mean value ± SE of triplicates is shown.

Table 1. Ratio of IL-10/IL-2 production from $CD4^+$ T cells stimulated with SEA and L cell transfectants as indicated

	Cell type	
Day	LDR7	LDR7/ICAM
3	12	0·2
5	13	1·2
7	>1500*	17
10	>1000*	48

*[IL-2] below detection limit (<0·5 U/ml).

lower, as compared to the IL-10/IL-2 ratio seen after LDR7 stimulation.

Increased expression of cell surface markers after ICAM-1 costimulation

Purified $CD3^+$ T cells stimulated with LDR7 and SEA expressed elevated levels of several cell surface activation markers including the IL-2Rα and β chains, CD71, CD11a, CD69, CD80 and the CD40L after 24 hs of culture and a further enhancement of these levels were seen after ICAM-1 costimulation (Fig. 3). In contrast, upregulation of the IL-2Rγ chain, CD11a and CD44 required ICAM-1 costimulation, because the levels of these molecules remained almost unaffected in the presence of the LDR7 single transfectant.

Monoclonal antibodies against LFA-1 or ICAM-1 efficiently blocked the IL-2 production in T cells stimulated with ICAM-1-transfected LDR7 cells.

In order to confirm a costimulatory role of ICAM-1 we added mAb against LFA-1 or ICAM-1 in order to block the costimulatory signal. As expected, addition of either anti-LFA-1 mAb or anti-ICAM mAb almost completely blocked the IL-2 production (Fig. 5), or proliferation (data not shown) from $CD4^+$ T cells costimulated with DR7/ICAM-1 cells. In contrast, the addition of a control immunoglobulin G (IgG) mAb (C215) had no effect.

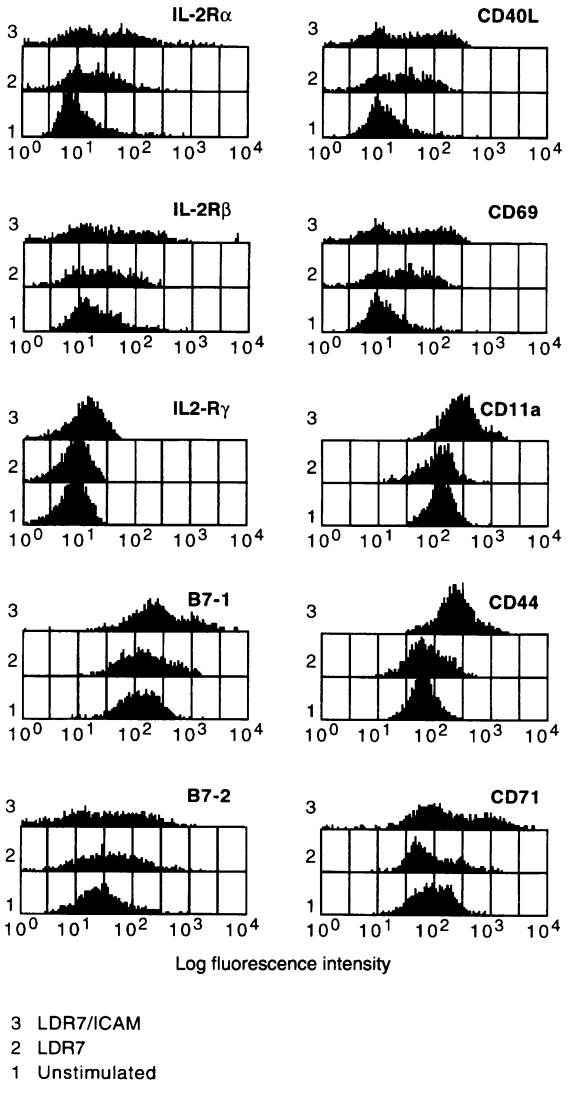

Figure 4. ICAM-1 induced upregulation of activation markers on $CD4^+$ and $CD8^+$ T cells. Flow cytometric analysis of expression of various activation markers, as indicated, following costimulation of $CD4^+$ or $CD8^+$ T cells for 24 hr in the presence of SEA (0·6 ng/ml). One out of two similar experiments is shown.

DISCUSSION

In this report we demonstrate a costimulatory role of ICAM-1 on SEA-activated $CD4^+$ and $CD8^+$ T cells, using mouse L cells transfected with ICAM-1 and/or MHC II (DR7), for presentation of SEA.

Previous studies have shown that IL-2Rα and γ chains are upregulated after B7.1 costimulation.[32] Our results show that T cells stimulated with HLA-DR single transfected L cells and SEA responded with upregulation of the IL-2Rα and β chains, whereas upregulation of the IL-2R-γ chain required ICAM-1 costimulation. This indicates that expression of high affinity IL-2R requires costimulation.

The proliferative response induced with ICAM-1 costimulation was accompanied by a distinct cytokine profile with significant IL-2 production in $CD4^+$ and $CD8^+$ T cells, reaching levels comparable to those seen in our laboratory after B7 costimulation of SEA activated $CD4^+$ T cells.[10] In contrast,

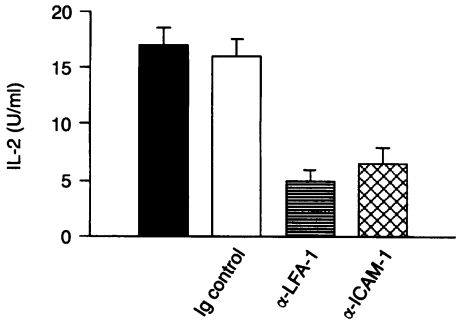

Figure 5. Anti-LFA-1 and anti-ICAM mAb inhibits ICAM-1-induced IL-2 production in $CD4^+$ T cells. Stimulation of $CD4^+$ T cells in the presence SEA (0·6 ng/ml) and LDR7/ICAM-1 transfectants. The T cells were preincubated with anti-LFA-1 mAb (IB4, 2·5 μg/ml) or L cell transfectants with anti-ICAM-1 mAb (1:1000) or control (C215 mAb, 5 μg/ml), respectively. One representative experiment out of three is shown.

lower levels of IFN-γ and TNF were detected in the ICAM-1 costimulated cultures as compared to the high levels seen after costimulation with B7 in our laboratory.[10] Recent studies have shown that ICAM-1 or B7 costimulation is equally sufficient at activating naive T cells to proliferation and IL-2 production[13] whereas co-operation between these two costimulatory pathways results in a synergistic effect[12,13,33] Our data suggest that ICAM-1 costimulation of SAg-triggered T cells results in a response characterized by high amplitude and short duration both in terms of proliferation and IL-2 production, whereas a sustained proliferative response with high levels of IL-2, IFN-γ and TNF requires the co-operation of several costimulatory molecules.[10]

Most interestingly, $CD4^+$ T cells stimulated with SEA, presented on LDR7 cells, produced significant levels of IL-10, whereas $CD4^+$ T cells costimulated with ICAM-1 released several-fold lower amounts of IL-10. In contrast, no IL-10 was produced by the $CD8^+$ T cells.

IL-10 inhibits proliferation and production of cytokines by leucocytes[34,35] and down-regulates the antigen-presenting function of APC,[36] thereby exerting repression of an immune response. IL-10 is produced from murine Th0 and Th2 type T helper cells and has been shown to exert a suppressive effect on IFN-γ and TNF production *in vivo* using IL-10 knock-out mice[37] or mAb neutralization experiments.[38] IL-10 has been proposed to be involved in the down-regulation of a Th1 type response, possibly by inhibiting IL-12 production from APC.[22]

Recent studies have proposed that immune responses driven predominantly by Th1 cells may play a central role in the pathogenesis of several organ-specific experimental autoimmune diseases in animals[39,40] and that resolution of the inflammation in these diseases might be associated with the differentiation of Th0 cells into Th2 cells.[41–43] The inhibitory role of IL-10 in an immune response indicates a possible beneficial role for immunoregulatory IL-10-producing T cells to down-regulate a Th1 type response at inflammatory sites, such as synovial joints of rheumatoid arthritis (RA) patients.[24] Moreover, LFA-1 has been reported to be expressed in an active configuration on synovial T cells[44] and recent studies suggests an active role for LFA-1 in the prevention of apoptosis of T cells.[45] Indeed, treatment of RA patients with anti ICAM-1 mAb have shown a beneficial therapeutic effect,[46,47] that correlates with increased T-cell hyporesponsiveness[46] and altered recruitment of activated Th1-like cells into the synovium.[48] These results together with our data showing a marked inhibitory role of ICAM-1 on IL-10 production from activated T cells imply that the beneficial effect of anti-ICAM-1 mAb in treatment of RA patients involves multiple mechanisms and it is tempting to suggest that, at least in part, this may be a result of increased IL-10 production from inflammatory T cells.

The proliferative response induced by the LDR7/ICAM-1 transfectants could be efficiently abrogated with mAb against LFA-1 or ICAM-1, showing that the proliferative response indeed is dependent on a functional ICAM-1–LFA-1 pathway. In contrast, an IgG control antibody had no effect on the ICAM-1-induced proliferation.

Several studies, including models for pulmonary inflammation[49] and arthritis[50] have shown a reduced inflammatory response in ICAM-1-deficient mice compared to wild type. However, the pattern of T-cell cytokines produced in ICAM-1 knock-outs following Ag challenge remains to be established.

In conclusion, our results show that ICAM-1 costimulation is sufficient for induction of a relatively short-lived proliferative response accompanied by high levels of IL-2 in SEA-activated T cells. Moreover, ICAM-1 costimulation seems to have an inhibitory role on IL-10 production from SEA-activated $CD4^+$ T cells resulting in a polarization towards a Th1 cytokine profile thereby favouring a pro-inflammatory response. Recent studies have shown a requirement for co-expression of ICAM-1 for rejection of B7 expressing tumours;[14] this makes it tempting to suggest that ICAM-1, in addition to increasing adhesion, may contribute by modifying the pattern of cytokines produced by the tumour-infiltrating lymphocytes. However, it remains to be investigated whether the inhibition of IL-10 production from SEA-stimulated human $CD4^+$ T cells is unique for ICAM-1 costimulation or if it is a common feature of several adhesive or costimulatory molecules.

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