mAb to specific epitopes (see Fig. 1), which revealed the presence of an immunodominant area of domain 1 to which five of the mAb bound, defined by the mutations K40/D (representing the substitution of aspartate for lysine at position 40) and L43LPGN/E-SPG (representing a four-amino-acid substitution commencing at position 43). In contrast, no binding to the areas defined by the mutants N56VQ/EIG, P36LP/QWL, or R49/W was seen in this series of antibodies.

Previous observations had shown that the L43LPGN/E-SPG mutation overlaps the *P. falciparum* binding site, allowing prediction as to which mAb should block the binding of *P. falciparum*-infected erythrocytes to ICAM-1. We therefore used a blinded panel of all 11 CD54 mAb to test their ability to inhibit binding of infected erythrocytes to ICAM-1, using a standardized adhesion assay with the parasite clone A4 binding to ICAM-1-Fc immobilized on plastic [8]. Neither the antibodies against domain 4, nor the domain 2 antibody S100 (8-4A6), showed any inhibition of parasitized erythrocyte binding (see Fig. 2). Two of the domain 1 antibodies, S095 (CBR-IC1/4) and S107 (RR1/1), also showed no inhibition of binding when compared to negative control antibodies. However, the five domain 1 mAb sensitive to the mutation defined by the L43LPGN/E-SPG mutation abolished infected cell binding (Fig. 2). Some or all of these mAb may also be expected to disrupt LFA-1 binding to ICAM-1 as this mutation also overlaps with that site.

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AS4.6 Characterization of the ICAM-3/LFA-1 interaction and its role in immune responses

ANTONIN R. DE FOUGEROLLES and TIMOTHY A. SPRINGER

Leucocyte adhesive interactions play an essential role in inflammatory and immune responses [1,2], and are mediated in part by LFA-1 (CD11a/CD18) and its ligands, ICAM-1 (CD54), ICAM-2 (CD102), and ICAM-3 (CD50) [2–5]. ICAM-3 (CD50) is a heavily glycosylated glycoprotein of M_r 124 000 that is well expressed on leucocytes and absent on endothelium [5,6] and that contains five Ig-like domains that are highly homologous to the corresponding domains in ICAM-1 and ICAM-2 [7–9].

A panel of six monoclonal antibodies (mAb) to human ICAM-3, S087–S092, were tested for their ability to inhibit adhesion of SKW3, an ICAM-3 bearing T-thymoma cell line, to purified LFA-1 and for their ability, in conjunction with ICAM-1 and ICAM-2 mAb, to inhibit phorbol myristate acetate (PMA)-induced aggregation of SKW3 cells (Fig. 1; Table 1). Two mAb S087 (CBR-IC3/1) and S092 (CBR-IC3/6), were capable of inhibiting ICAM-3 interactions with purified LFA-1 (Fig. 1). Blocking with CBR-IC3/1 and CBR-IC3/6 was partial when used alone, and essentially complete when used with mAb to ICAM-1 and ICAM-2, consistent with expression by SKW3 cells of all three ICAMs [5].

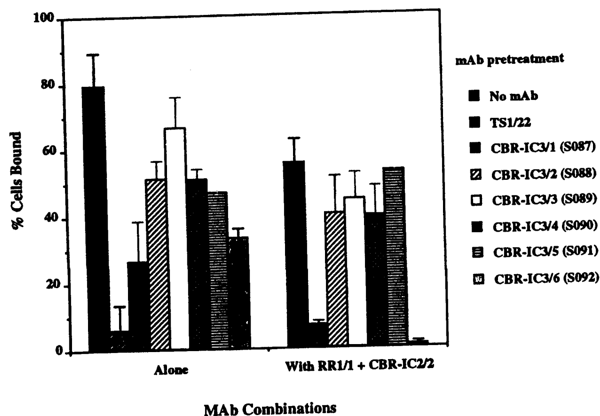

Fig. 1 Effect of anti-ICAM-3 mAb on adhesion of SKW3 cells to purified LFA-1. Purification of LFA-1 and adhesion of cells was as previously described [4]. Cells were pre-treated with saturating concentrations of one of six ICAM-3 mAb (S087–S092) in the presence or absence of blocking ICAM-1 (RR1/1) and ICAM-2 (CBR-IC2/2) mAb and allowed to bind to LFA-1-coated microtitre wells for 60 min at 37 °C and then washed six times by aspiration. Alternatively, the absorbed purified LFA-1 was pre-treated with LFA-1α mAb (TS1/22). Site density of LFA-1 as determined by radioimmunoassay was 700 sites/μm^2. One representative experiment of three is shown and bars show one standard deviation.

None of the ICAM-3 mAb were capable of inhibiting PMA-induced aggregation by themselves or in combination with ICAM-1 and ICAM-2 mAb (Table 1). Aggregation was, however, completely inhibited when certain combinations of ICAM-3 mAb were used in conjunction with ICAM-1 and ICAM-2 mAb (Table 1). One such example was when mAb S088 (CBR-IC3/2) was used in conjunction with mAb S087 (CBR-IC3/1) (Table 1). Interestingly, S087 (CBR-IC3/1) and S092 (CBR-IC3/6) were capable of inducing aggregation of SKW3 cells and of CD18-deficient SLA B-cells, in a manner that was cation- and temperature-dependent, and inhibitable only with ICAM-3 mAb (that is, S088 (CBR-IC3/2)) recognizing different epitopes; mAb to other adhesion structures, including VLA (α_{1-6}), CD29 (β_1), LFA-1, Mac-1, p150,95, CD18 (β_2), CD2, LFA-3, β_4, and β_7, had no effect on this aggregation (data not shown).

To test whether the avidity of LFA-1 for ICAM-3 could be regulated by PMA, as has been previously demonstrated for ICAM-1, cells treated with or without PMA were assayed for binding to purified ICAM-1 and ICAM-3 (Fig. 2(A)). Binding to ICAM-3 was enhanced fivefold by PMA stimulation, while binding to ICAM-1 was also enhanced, as previously reported [10,11]. Adhesion was specific as it was inhibited by the pre-treatment of cells with an LFA-1 mAb, TS1/22 (Fig. 2(A), (B)). Similarly, adhesion to purified ICAM-3 could be inhibited with mAb to ICAM-3 (Fig. 2(B)). Interestingly, adhesion was not blocked with either mAb S087 (CBR-IC3/1) or S088 (CBR-IC3/2) alone, but only when the two were used together. As had been previously shown for ICAM-1/LFA-1 interactions [12], adhesion to purified ICAM-3 was also temperature- and cation-dependent (data not shown). Lastly, purified ICAM-3, when co-immobilized with CD3 mAb, provided a strong costimulatory signal for T-cell proliferation in a manner similar to that reported for ICAM-1 (13,14) and ICAM-2 (15) (data not shown).

Table 1 Effect of ICAM-3 mAb on PMA-induced SKW3 aggregation in the presence of ICAM-1 and ICAM-2 mAb

Workshop mAb		Effect on PMA-induced SKW3 aggregation of ICAM-3 mAb*					
Code	Clone name	CBR-IC3/1	CBR-IC3/2	CBR-IC3/3	CBR-IC3/4	CBR-IC3/5	CBR-IC3/6
S087	CBR-IC3/1	3	0	0	0	0	5
S088	CBR-IC3/2		3	3	1	3	0
S089	CBR-IC3/3			3	3	3	1
S090	CBR-IC3/4				2	3	2
S091	CBR-IC3/5					3	1
S092	CBR-IC3/6						5

*SKW3 cells were pre-incubated at room temperature for 45 min with one or combinations of any two ICAM-3 mAb in the presence of ICAM-1 mAb S107 (RR1/1) and ICAM-2 mAb S086 (CBR-IC2/2). Cells were stimulated with 50 ng/ml PMA and aggregation scored visually after 5 h. Scores: 0, no cells clustered; 1, less than 10% of cells aggregating; 2, 10–50% of cells aggregating; 3, 50–100% of cells aggregating; 4, nearly 100% of cells in loose aggregates; 5, 100% of cells in very compact aggregates. Cells aggregated in the absence of mAb (score 4) and in the presence of only ICAM-1 and ICAM-2 mAb (score 3). Aggregation was not inhibited by ICAM-3 mAb alone (score 4). LFA-1 mAb completely inhibited aggregation (score 0). One of two representative experiments is shown.

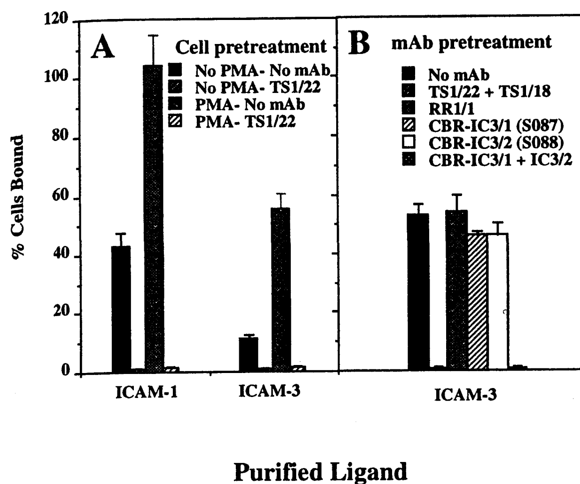

Fig. 2 Binding of PMA-stimulated SKW3 cells to purified ICAM-1 and ICAM-3. (A) SKW3 cells were incubated in the presence of either no mAb or mAb TS1/22 (anti-LFA-1α), and allowed to bind in the presence or absence of 50 ng/ml PMA to Petri plates spotted with purified ICAM-1 and ICAM-3 as previously described [16–18]. After 1 h at 37 °C, unbound cells were removed by six washes with a transfer pipette, and the percentage of cells bound was quantitated by visually scoring the number of cells in six microscopic fields (100× magnification). (B) SKW3 cells were incubated in the presence of either no mAb or mAb TS1/22 and TS1/18 (anti-LFA-1α and anti-CD18, respectively), or, alternatively, the absorbed purified ICAM-1 and ICAM-3 was pretreated with either mAb RR1/1 (anti-ICAM-1), mAb CBR-IC3/1 (anti-ICAM-3), mAb CBR-IC3/2 (anti-ICAM-3), or mAb CBR-IC3/1 and CBR-IC3/2 (anti-ICAM-3). Cells were then allowed to bind in the presence of 50 ng/ml PMA to spots of purified ICAM-1 and ICAM-3 as described above. One of three representative experiments is shown and bars show one standard deviation.

To test the hypothesis that ICAM-3 plays an important role in the initiation of immune responses, the relative importance of each ICAM, separately and in various combinations, was tested in a variety of immunological assays. mAb to all three ICAMs were tested for their ability to inhibit hepatitis B surface antigen (HBsAg)-dependent proliferation of peripheral blood lymphocytes (PBL) from hepatitis B-vaccinated, responding individuals (Fig. 3). Proliferation was inhibited by LFA-1 mAb to levels seen in unstimulated PBL. ICAM-1, -2, and -3 mAb showed slight inhibition when used separately; however, a combination of mAb to all three ICAMs reduced proliferation to levels seen in unstimulated PBL. Similarly, blocking mAb to all three ICAMs inhibited proliferative response of lymphocytes to phytohaemagglutinin (PHA) and allogeneic stimulator cells (data not shown).

The ability of a combination of mAb to ICAM-1, ICAM-2, and ICAM-3 to inhibit LFA-1-dependent adhesion of lymphoid cell lines, such as SKW3, and different proliferative responses to the same extent as done by mAb to LFA-1 suggests that the most functionally important, and possibly all, of the ICAMs expressed on lymphocytes have now been defined.

Acknowledgement

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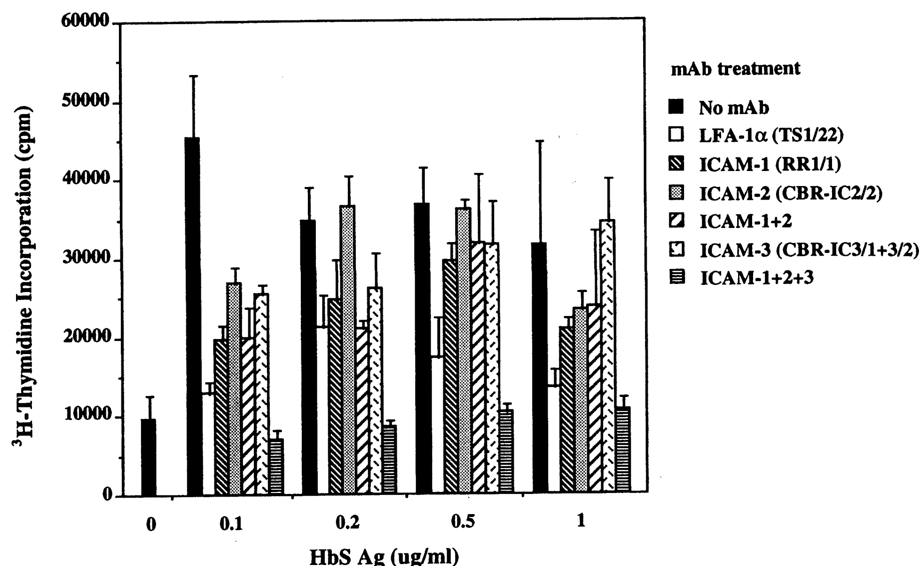

Fig. 3 Inhibition of antigen-specific proliferation by LFA-1, CD18, ICAM-1, ICAM-2, and ICAM-3 mAb. Proliferation of PBL from vaccinated responding individuals in response to recombinant hepatitis B surface antigen (HbsAg) was as previously described [19]. mAb were added at the start of the cell incubations at final mAb concentrations of 20 μg/ml. mAb used were to LFA-1 (TS1/22), CD18 (TS1/18), ICAM-1 (RR1/1), ICAM-2 (CBR-IC2/2), and ICAM-3 (S087 (CBR-IC3/1) and S088 (CBR-IC3/2)). One of three representative experiments is shown and bars show one standard deviation.

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AS4.7 CD50 (ICAM-3) regulates both intercellular T-cell adhesion and activation

MIGUEL R. CAMPANERO, MIGUEL A. DEL POZO, PALOMA SÁNCHEZ-MATEOS, ALICIA G. ARROYO, and FRANCISCO SÁNCHEZ-MADRID

The interaction of integrins with their ligands is thought to mediate intercellular signalling events that regulate cellular functions [1]. In this regard, the interaction of the leucocyte integrin LFA-1 with one of its ligands, ICAM-1, is able to provide stimulatory signals for activation of human T cells [2]. However, little is known about the regulatory signals that the integrin counterreceptors can mediate. A third ligand for the LFA-1 integrin, ICAM-3 (CD50), has recently been identified and cloned [3–6]. In contrast to