Engagement of Major Histocompatibility Complex Class II Molecules Induces Sustained, Lymphocyte Function–associated Molecule 1–dependent Cell Adhesion

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Summary

Antigenic stimulation is associated with enhanced adhesion between T cells and antigen-presenting cells (APC). Binding of ligands to the T cell antigen receptor activates the adhesion function of lymphocyte function–associated molecule 1 (LFA-1; CD11a/CD18). We demonstrate here that ligand binding to major histocompatibility complex class II (Ia) molecules also activates LFA-1 function, providing a reciprocal mechanism for the induction of adhesion between T cells and Ia$^+$ APC. Adhesion was affected by a qualitative change in LFA-1 molecules and was reversed by the protein kinase C inhibitor sphingosine. These results define a novel role for Ia molecules as signal transducing receptors that regulate LFA-1-dependent adhesion via a putative, Ia-coupled protein kinase(s).

Cognate recognition of APC by antigen-specific T cells is critically dependent on adhesive interactions mediated by LFA-1 molecules present on both T cells and APC (1). While LFA-1 is constitutively expressed on lymphocytes, its adhesion function is activated after antigen-specific recognition (2). Engagement of the TCR by mAbs was recently shown to upregulate the affinity of LFA-1 for its counter receptors (3, 4). This proceeds via intracellular signaling mechanisms that involve the activation of protein kinase C (PKC). We demonstrate here that Ia molecules can similarly transduce intracellular signals that activate LFA-1 molecules on Ia$^+$ cells.

Materials and Methods

Reagents and Antibodies. Toxic shock syndrome toxin 1 (TSST-1) and Staphylococcal exotoxin B (SEB) were obtained from Toxin Technology (Madison, WI). mAb ST259 was kindly provided by Dr. E. Yunis (Dana Farber Cancer Institute, Boston, MA). mAb L243, directed against monomorphic determinants on human HLA-DR molecules, was generated from hybridoma cells (American Type Culture Collection, Bethesda, MD). Anti-LFA-1α chain mAb IOT16, anti-LFA-1β chain mAb IOT18, and anti-ICAM-1 mAb 84H10 were all obtained from AMAC, Inc. (Westbrook, ME). Sphinghosine, ceramide, BSA, and PMA were obtained from Sigma Chemical Co. (St. Louis, MO).

Cells. High density tonsillar human B cells and resting human T cells were isolated as described (5). Activated T cells were prepared by stimulating resting T cells with PHA for 5 d, followed by stimulation with IL-2 at 10 U/ml for an additional 5 d. EBV-transformed lymphoblastoid B cell lines were derived from normal donors and from an immunodeficient patient with absent Ia expression. Monocytes were prepared as described (6).

Aggregation Assays. Cells were resuspended in RPMI 1640 and seeded in 96-well microtiter plates at 2 × 10⁵ cells/well for B cells, monocytes, and T cells, and at 5 × 10⁴ for lymphoblastoid B cell lines. Cells were stimulated at 37°C for the indicated time periods with TSST-1 at 1 μg/ml; SEB at 10 μg/ml; Fab mAb L243 at 1 μg/ml; Fab mAb ST2-59 at 1 μg/ml, or with PMA at 25 μg/ml. Cell aggregation was monitored using light microscopy by counting the number of cell aggregates containing ⩾10 cells per well (7). Values are means of scores of two wells ± SD, and the data are representative of three other experiments.

Results and Discussion

Fig. 1 *A* demonstrates that *Staphylococcus* TSST-1, which binds to Ia molecules (8), induces the aggregation of Ia$^+$ human tonsilar B cells. Aggregation was evident by 15 min post-stimulation, involved ⩾80% of the cells in culture by 4 h, and was sustained for at least 12 h. Identical aggregation was obtained with the Fab fragment of mAb L243, which recognizes an epitope on HLA-DR closely related to the TSST-1 binding site (8). Aggregation induced by TSST-1 and by mAb L243 was similar in magnitude to that induced by the PKC activator PMA, which affects sustained activation of LFA-1 adhesion function (3, 9). Two ligands that bind to epitopes distinct from those recognized by TSST-1 and by mAb L243, namely SEB and the Fab fragment of the anti-HLA-DR/DQ mAb ST2-59 (8, 10), failed to induce B cell
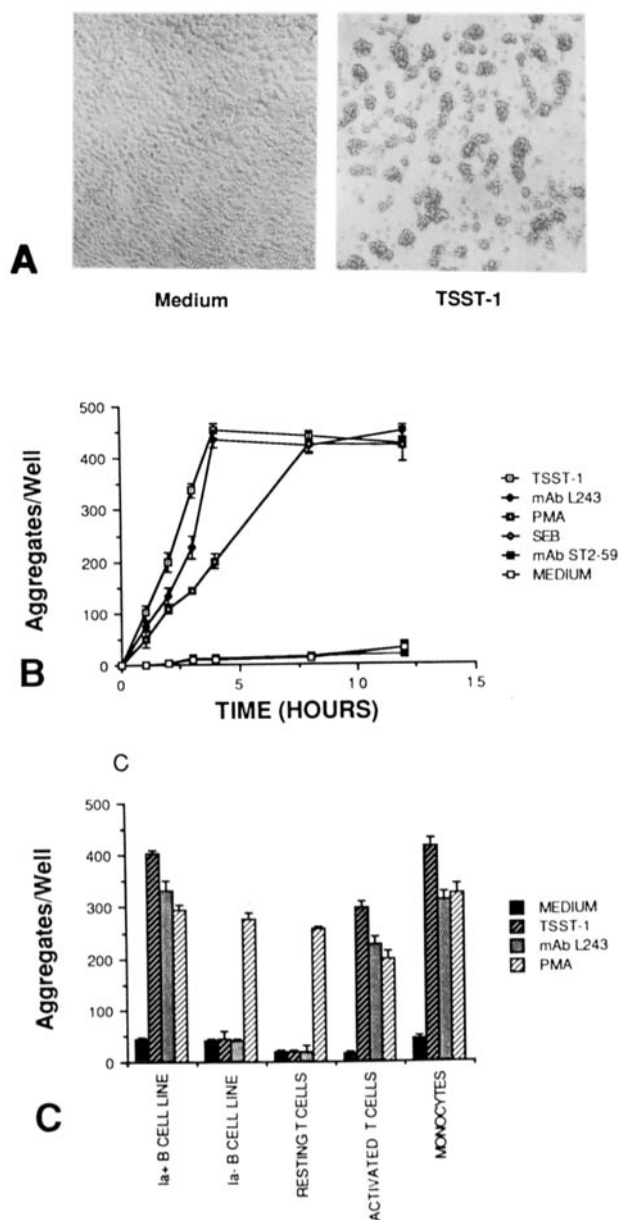
express Ia molecules on their surface, or of B lymphoblastoid cells derived from a patient with congenital absence of Ia expression (11) (Fig. 1 C).

Adhesion induced by Ia-binding ligands was mediated by LFA-1 and its counter receptors. mAbs to either the α chain or the β chain of the LFA-1 heterodimer inhibited cell aggregation induced by TSST-1 or by mAbs L243 (Fig. 2 A), as well as by PMA, as previously noted (9). In addition, a mAb directed against intercellular adhesion molecule 1 (ICAM-1), a counter receptor for LFA-1 (12), partially inhibited cell aggregation induced by TSST-1 and by mAb L243 (Fig. 2 A). The more effective inhibition of cell aggregation by anti-LFA-1 mAbs compared with anti-ICAM-1 mAb suggests that other LFA-1 counter receptors, such as ICAM-2 (13), play a role in Ia-triggered adhesion. Ia-mediated adhesion was less
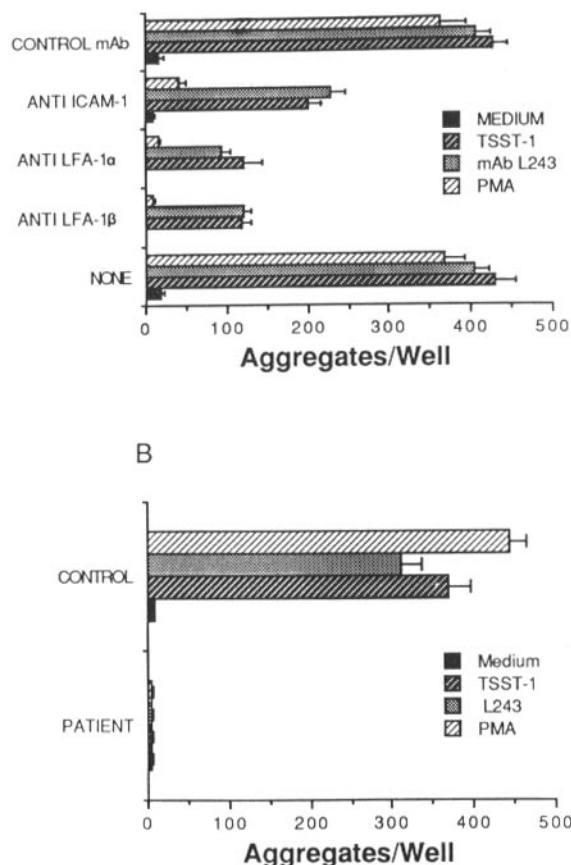
Figure 1. Induction of leukocyte adhesion by Ia-binding ligands. (A) Photomicrographs of tonsillar human B cells treated for 4 h with medium alone or with TSST-1. (B) Time course of aggregation of tonsillar B cells induced by Ia-binding ligands. (C) Ia-binding ligands induce the aggregation of Ia+ but not of Ia− leukocytes. (C) Resting T cells and monocytes were stimulated for 4 h while activated T cells and B lymphoblastoid cell lines were stimulated for 1 h.

Figure 2. Induction of adhesion via Ia molecules is mediated by LFA-1 and its counter receptors. (A) Inhibition of aggregation of high density tonsillar B cells by anti-LFA-1α chain mAb, anti-LFA-1β chain mAb, anti-ICAM-1 mAb, and by a control mAb, ST2-59. All mAbs were added at a final concentration of 25 μg/ml. (B) Induction of adhesion in monocyte and B lymphocyte–enriched preparations of PBMC from a normal donor or from a patient with leukocyte adhesion deficiency. PBMC were enriched for monocytes and B cells by rosetting with SRBC and collecting the nonrosetting fraction, as described (5). (A and B) Cells were treated for 4 h with the indicated stimulus, as described for Fig. 1. Results are representative of a total of three experiments for A and two experiments for B.
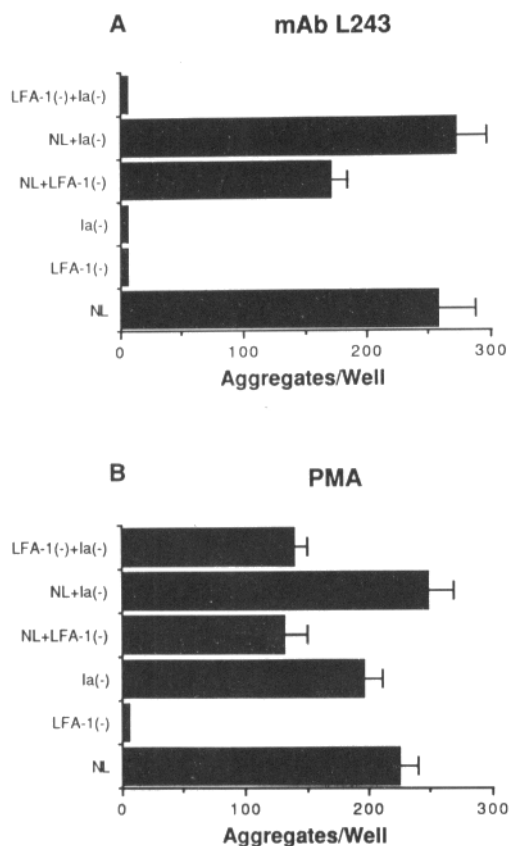
aggregation. Thus, induction of adhesion via Ia is restricted by the epitope specificity of the binding ligands.

TSST-1 and mAb L243 induced the aggregation of other Ia+ leukocytes, including activated T cells, monocytes, and B lymphoblastoid lines. The requirement for Ia surface expression for the induction of cell aggregation by TSST-1 and mAb L243 was demonstrated by the failure of both ligands to induce the aggregation of resting T cells, which do not

A   mAb L243

B   PMA

Figure 3. Ia ligands induce cell aggregation by activating the adhesion function of LFA-1 and not that of its counter receptors. T cell lines derived from a normal donor [NL], from a leukocyte adhesion deficiency patient [LFA-1(−)], or from an Ia-deficient patient [Ia(−)] were propagated with PHA, IL-2, and irradiated allogeneic feeder cells as described (17). All three lines expressed comparable levels of ICAM-1 and, when applicable, comparable levels of LFA-1 and Ia. Homogenous cell populations were seeded at 2×10^5 cells/well, while mixtures of two cell populations were seeded at 10^5 cells/well for each cell type, and the cells were treated with respective stimulus for 6 h. To ascertain heterotypic cell adhesion in mixture of different T cell populations, cells from population were first loaded with the dye 6 carboxyfluorescein diacetate at 100 μM for 1 h at 37°C, washed extensively, and then mixed with the respective cell type, stimulated for 6 h, and then examined by light and fluorescence microscopy. Similar results were obtained in one other experiment.

amenable to disruption by mAbs to LFA-1 and ICAM-1 as compared with PMA-induced adhesion. This may relate to a higher affinity interaction between LFA-1 and ICAM in cells activated via Ia or due to the involvement of other adhesion molecules in Ia-mediated adhesion. The critical role of LFA-1 in mediating adhesion induced by Ia-binding ligands was ascertained by studying a patient whose leukocytes were severely defective in the expression of LFA-1 (<2% of normal) (14). Fig. 2 B demonstrates that TSST-1 and mAb L243 induced adhesion in normal but not in LFA-1-deficient PBMC preparations enriched in B lymphocytes and in monocytes. Finally, this cell aggregation was Mg^{2+} and temperature dependent, typical of LFA-1-mediated adhesion interactions (9) (data not shown).
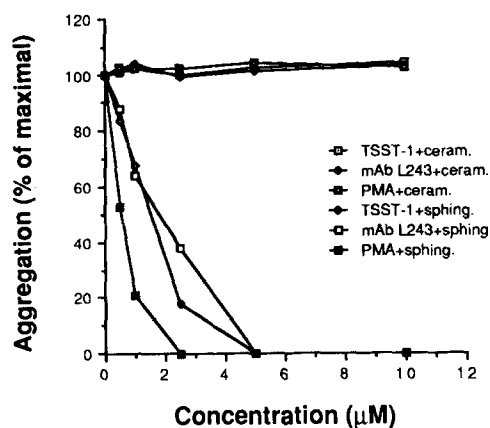

Figure 4. Inhibition of TSST-1-, Fab mAb L243-, and PMA-induced adhesion by sphingosine. Tonsillar human B cells were stimulated in the absence or in the presence of sphingosine or the sphingosine metabolite ceramide, which were added as a 1:1 complex with fatty acid–free BSA. Results are presented as percent ratio of aggregation observed for each stimulus in the presence of sphingosine or ceramide compared with aggregation observed in the absence of sphingosine or ceramide.

Adhesion induced by Ia-binding ligands was not associated with upregulation of the surface expression of LFA-1 or of ICAM-1, and did not require protein synthesis, as it was not affected by cycloheximide, an inhibitor of protein synthesis (data not shown). These results suggested that Ia-induced adhesion is effected by a qualitative change in LFA-1 and/or its counter receptors.

Heterolytic adhesion experiments using primary T cell lines derived from normal donors, from the LFA-1-deficient patient, and from the Ia-deficient patient demonstrated that Ia-induced adhesion was affected by activating LFA-1 adhesion function and not by activating the adhesion function of LFA-1 counter receptors. Fig. 3 A demonstrates that mAb L243 failed to elicit either homotypic or heterolytic aggregates in mixtures of LFA-1-deficient and Ia-deficient activated T cells. In contrast, PMA induced vigorous heterotypic aggregation in the same cell mixtures (Fig. 3 B). Both mAb L243 and PMA induced heterolytic aggregation in mixtures of Ia^+ T cells and LFA-1-deficient cells, and in mixtures of Ia^+ T cells and Ia-deficient T cells (Fig. 3).

PKC activators may enhance LFA-1 function by inducing the phosphorylation of LFA-1β chain (6) or of cytoskeletal proteins that interact with LFA-1, such as talin (15). To determine the role of PKC in Ia-mediated adhesion, we examined the capacity of the PKC inhibitor sphingosine (16) to antagonize the induction of adhesion by Ia-binding ligands. Fig. 4 A demonstrates that sphingosine inhibited the aggregation of tonsillar B cells induced by TSST-1, by mAb L243, or by PMA. In contrast, the inert sphingosine metabolite ceramide exhibited no effect on cell aggregation. Other protein kinase inhibitors such as stauporine and H7 also inhibited cell aggregation (data not shown). These results suggested that an Ia-coupled protein kinase, either PKC itself or a closely related enzyme, mediates the induction of adhe-

sion via Ia molecules. Other experiments established that Ia-mediated adhesion was not mimicked by activators of Ca^{2+}/calmodulin-dependent or cAMP-dependent protein kinases. The identity of the protein kinase mediating the induction of adhesion via Ia is currently under investigation.

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