Staphylococcal enterotoxin B-specific adhesion of murine splenic T cells to a human endothelial cell line

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SUMMARY

The presence of a putative autoantigen of autoimmune disorder in a target organ may cause accumulation of specific T cells in the inflammatory region. One of the mechanisms of such accumulation involves the migration of specific-circulating T cells through the endothelial cells into the target lesion. The presence of only a few specific T cells responsive to a putative autoantigen has hampered the investigation of specific migration of circulating T cells to the target organ. We used a superantigen to investigate specific T-cell adhesion to endothelial cells, because it stimulates a large proportion of T cells with particular $V\beta$ elements and adhesion of T cells to the endothelium is a vital step in the migration process. Adhesion of murine T cells to the human endothelial cell line, EA.hy926, was specifically increased in the presence of staphylococcal enterotoxin B (SEB). The increase was interferon-γ (IFN-γ)-dependent, and consisted mainly of $CD4^+$ T cells. $V\beta8.1,2^+$ T cells preferentially adhered to endothelial cells in the presence of SEB compared with $V\beta6^+$ T cells. Pretreatment of endothelial cells with SEB increased the adherence of $V\beta8.1,2^+$ T cells, while anti-human leucocyte antigen (HLA)-DR and -DQ antibodies inhibited the increased adherence of $V\beta8.1,2^+$ T cells. Our results demonstrate that increased T-cell adhesion to endothelial cells is SEB specific, and that the specificity is dependent on major histocompatibility complex (MHC) class II molecules expressed on endothelial cells and on the recognition of the SEB–MHC class II complex by $V\beta8.1,2^+$ T cells.

INTRODUCTION

In autoimmune diseases such as rheumatoid arthritis (RA) and multiple sclerosis (MS), T cells are important for the auto-immune processes, as demonstrated in animal models such as collagen-induced arthritis and experimental autoimmune encephalomyelitis (EAE).[1,2] Accumulation of encephalitogenic T cells in the central nervous system (CNS) has also been demonstrated.[3] Although a biased T-cell receptor (TCR) Vα or Vβ expression in the synovial fluid or tissue of RA is still controversial,[4–10] accumulation of T cells expressing a particular TCR Vβ and Vα has been reported in the RA synovial fluid or tissue.[4–7,10] Similarly, CNS accumulation of T cells that are activated by myelin basic protein occurs in patients with MS.[11] Thus, accumulation of T cells bearing particular TCR

Vβ families in the affected lesion suggests a pathoaetiological role for superantigens in RA and MS.[4,6,12]

Several mechanisms may enhance the prevalence of a particular T cell in a pathological lesion, including preferential migration, preferential retention and preferential proliferation. Adhesion of T cells to endothelial cells is an important process in the migration of circulating T cells to the target tissue. The aim of the present study was to determine whether the increase in a particular T-cell type can be induced by a preferential migration caused by antigen stimulation. Therefore, the specific adhesion of T cells to endothelial cells was investigated using staphylo-coccal enterotoxin B (SEB), which is one of the superantigens known to activate murine T cells expressing the TCR V$\beta8$ element. Our results demonstrated a preferential adhesion of murine $V\beta8.1,2^+$ T cells to a human endothelial cell line in the presence of SEB *in vitro*. SEB increased the proportion of adherent T cells, particularly $CD4^+$ cells. Furthermore, the process was dependent on the expression of human leucocyte antigen (HLA)-DR and -DQ on endothelial cells. Anti-intracel-lular adhesion molecule-1 (ICAM-1) monoclonal antibody (mAb) inhibited the enhanced adhesion of $V\beta8.1,2^+CD4^+$ T cells, but anti-vascular cell adhesion molecule-1 (VCAM-1) mAb did not. Our results indicate that circulating T cells specific to the antigen presented on the endothelial cells in the target organ can attach preferentially to the endothelium.

MATERIAL AND METHODS

Preparation of T cells

The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation at our institution. BALB/c mice were obtained from Charles River (Yokohama, Japan), and maintained in the animal facility of Nagasaki University School of Medicine. At 2–3 months of age, the animals were killed under diethylethel anaesthesia and the spleen dissected, removed and a single cell suspension prepared in phosphate-buffered saline (PBS) containing 1% heat-inactivated fetal bovine serum (FBS; Equitech-Bio, Ingram, TX). After washing, Tris-buffered 0·16 M NH$_4$Cl was added for lysis of red blood cells (RBC). Spleen cells were plated onto a tissue culture dish (Becton Dickinson, Lincoln Park, NJ) precoated with 10 ml of 40 μg/ml affinity-purified F(ab′)$_2$ fragment of goat anti-mouse immunoglobulin (Cappel, Durham, NC), and incubated at 37° in 5% CO$_2$/humidified air for 60 min. Non-adherent cells were collected by washing with PBS containing 10% FBS and resuspended in 10 ml of RPMI-1640 culture medium (Gibco, Paisley, UK) containing 10% FBS supplemented with 100 U/ml penicillin (Meiji Seika, Tokyo, Japan), 120 mg/l streptomycin sulphate (Gibco), 300 mg/l L-glutamic acid (Sigma, St Louis, MO), 110 mg/l pyruvic acid (Sigma) and 2 mg/l NaHCO$_3$ (complete medium). More than 85% of the non-adherent spleen cells were CD3$^+$ cells, as assessed by flow cytometry (FCM).

Endothelial cells

The human endothelial cell line, EA.hy926, was a kind gift from Dr C.-J. S. Edgell (Department of Pathology, University of North Carolina School of Medicine, NC). The cell line was maintained in complete medium and plated onto a 6-well tissue culture cluster (Costar, Cambridge, MA) at a concentration of 2 × 10^5/well, and cultured for 3 days with or without 1000 IU/ml interferon-γ (IFN-γ) (Shionogi Co., Osaka, Japan).

Antigens and mAb

SEB and staphylococcal enterotoxin A (SEA) were purchased from Sigma. B-cell hybridomas producing antibodies against murine Vβ8.1,2 mAb (KJ16–133) were a kind gift from Dr J. Kappler (Howard Hughes Medical Institute, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO), and against Vβ6 mAb (RR4-7) were a kind gift from Dr M. J. Bevan (Howard Hughes Medical Institute, Department of Immunology, University of Washington, Seattle, WA). Fluorescein isothiocyanate (FITC)-conjugated goat anti-rat IgG was purchased from Tago (Camarillo, CA). Phycoerythrin (PE)-conjugated anti-murine CD4 and PE-conjugated anti-murine CD8 mAb were purchased from Cedarlane (Hornby, Canada). Anti-human ICAM-1, VCAM-1 and HLA-DR mAb were purchased from Immunotech (Marseille, France). Monoclonal antibodies against human HLA-DQ and murine lymphocyte function-associated antigen-1α (LFA-1α) were purchased from Sigma and Endogen (Cambridge, MA), respectively.

Fluorescence-activated cell sorter (FAC) analysis

Adherent T cells were incubated with anti-Vβ8.1,2 mAb or anti-Vβ6 mAb for 30 min on ice. After washing twice, the cells were exposed to FITC-conjugated goat anti-rat IgG and incubated again for 30 min on ice. Cells were then washed three times and incubated with PE-conjugated anti-CD4 and CD8 mAb. Stained cells were analysed by an Epics Profile II flow cytometer (Coulter Electronics, Hialeah, FL).

Preparation of adherent T cells to the endothelial cell line

T cells (2 × 10^6/well) were co-cultured with EA.hy926 cells in the presence or absence of SEB (1·0 μg/ml). After incubation for 120 min at 37° in 5% CO$_2$, the wells were washed 10 times with PBS containing 1% FBS to remove completely non-adherent lymphocytes. Adherent cells were then collected after treatment with 0·265 mM EDTA for 5 min at 37° in 5% CO$_2$. Sample analysis was performed in triplicate in each experiment. Each experiment was performed more than twice, and representative data are presented.

Statistical analysis

Data are expressed as mean ± SD. Differences between two sets of experiments were evaluated using the Student's two-tailed unpaired t-test. A P level of <0·05 was considered statistically significant.

RESULTS

Effect of SEB on number and percentage of adherent CD4$^+$ T cells

T cells adherent to endothelial cells pretreated with IFN-γ were collected and the number of viable cells was counted by trypan blue dye exclusion. As shown in Fig. 1a, SEB significantly increased the total number of adherent T cells from a control of 0·15 × 10^4 ± 0·05 × 10^4 to 1·32 × 10^4 ± 0·21 × 10^4 (P < 0·01). The subset type of adherent T cells was analysed with anti-CD4 and anti-CD8 mAb. SEB significantly increased the percentage of CD4$^+$ T cells among adherent T cells compared with the control conditions, but did not influence that of CD8$^+$ T cells (Fig. 1b). Thus, CD4$^+$ cells were the predominant type of
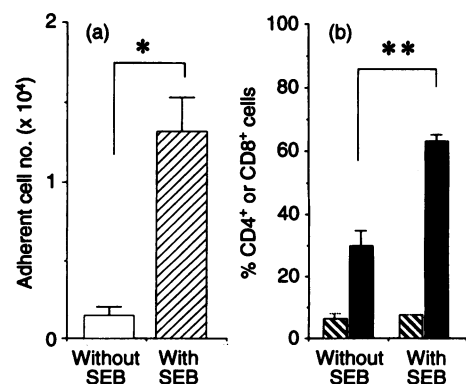

Figure 1. Effect of SEB on number and percentage of adherent CD4$^+$ T cells. Murine spleen T cells were placed on the IFN-γ endothelial cell line, EA.hy926, for 2 hr. After extensive washing, the number of adherent T cells was counted and the percentage of CD4$^+$ or CD8$^+$ cells analysed by flow cytometery. (a) The absolute number of murine spleen cells adherent to the endothelial cells. Open bars, without SEB; hatched bar, with SEB. (b) Percentage of CD4$^+$ or CD8$^+$ cells. Hatched bars, CD8$^+$ cells; closed bars, CD4$^+$ cells. The data are representative of two experiments. *P < 0·01, **P < 0·001.
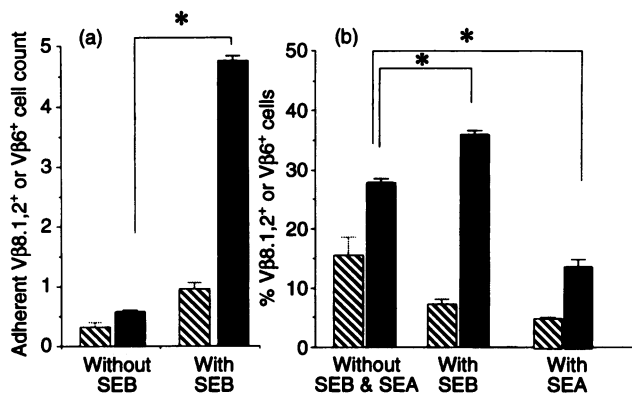
Figure 2. Effect of SEB on the adherent cell count and the percentage of Vβ8.1,2$^+$ or Vβ6$^+$ cells. (a) Adherent Vβ8.1,2$^+$ and Vβ6$^+$ cell count with or without SEB. (b) The percentage of adherent CD4$^+$ Vβ8.1,2$^+$ and CD4$^+$ Vβ6$^+$ cells among CD4$^+$ cells with or without SEB and SEA. Hatched bars, Vβ6$^+$ cells; closed bars, Vβ8.1,2$^+$ cells. The data are representative of two experiments. *P < 0·001.

T cells adhering to the endothelial cells in the presence of SEB.

TCR Vβ expression of adherent T cells

The percentages of CD4$^+$ TCR Vβ8.1,2$^+$ T cells, known as the TCR Vβ family member to be activated by SEB, and that of CD4$^+$ TCR Vβ6$^+$ T cells, among adherent CD4$^+$ T cells, were analysed using flow cytometry. As shown in Fig. 2, the absolute number of adherent Vβ8.1,2$^+$ T cells as well as the relative number of CD4$^+$ Vβ8.1,2$^+$ T cells increased significantly in the presence of SEB (control 27·9 ± 0·7%; SEB 36·0 ± 0·6%, P < 0·0001). On the other hand, while the number of Vβ6$^+$ T cells increased in the presence of SEB, albeit insignificantly (Fig. 2a), the percentage of CD4$^+$ Vβ6$^+$ T cells decreased from a control of 15·7 ± 3·0% to 7·4 ± 0·7% in the presence of SEB (Fig. 2b). The percentage of CD4$^+$ Vβ8.1,2$^+$ and CD4$^+$ Vβ6$^+$ T cells among adherent CD4$^+$ T cells under control conditions was similar to that in fresh spleen cells before the adherent assay, indicating a lack of a preferential adhesion of TCR Vβ8.1,2$^+$ T cells or Vβ6$^+$T cells to endothelial cells in the absence of SEB. Furthermore, the addition of SEA (1·0 μg/ml) significantly decreased the percentage of CD4$^+$ Vβ8.1,2$^+$ to 13·9 ± 1·1%, and that of CD4$^+$ Vβ6$^+$ to 5·1 ± 0·2%. This finding suggested that preferential adhesion of T cells bearing certain TCR Vβs other than Vβ8.1,2 and Vβ6, which are responsive to SEA, might occur in the presence of SEA (Fig. 2b).

Effect of IFN-γ on the percentage of adherent CD4$^+$ Vβ8.1,2$^+$ T cells

IFN-γ significantly increased the percentage of CD4$^+$ Vβ8.1,2$^+$ T cells adherent to the endothelial cells, from a control of 23·7 ± 3·6% to 31·7 ± 5·8% (P < 0·01; Fig. 3). IFN-γ is known to augment major histocompatibility complex (MHC) class II molecule expression on the cell surface, and binding of SEB to MHC class II antigen is a prerequisite for T-cell activation. Therefore, the augmentation of MHC class II antigen expression on endothelial cells may be an important factor for the increased
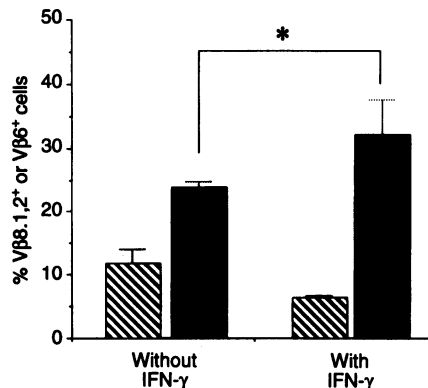

Figure 3. Effect of SEB on the percentage of CD4$^+$ Vβ8.1,2$^+$ or CD4$^+$ Vβ6$^+$ cells among adherent CD4$^+$ cells with or without treatment of endothelial cells with IFN-γ. Without IFN-γ treatment, the percentage of adherent Vβ8.1,2$^+$ cells and Vβ6$^+$ cells was at a similar level to that of Vβ8.1,2$^+$ cells and Vβ6$^+$ cells of suspended spleen T cells. Hatched bars, Vβ6$^+$ cells; closed bars, Vβ8.1,2$^+$ cells. The data are representative of two experiments. *P < 0·01.

CD4$^+$ Vβ8.1,2$^+$ T-cell adhesion in the presence of SEB. In fact, culture of cells in the presence of IFN-γ for 3 days vigorously augmented the surface expression of HLA-DR antigen on the endothelial EA.hy926 cell line (data not shown).

Preincubation of endothelial cells with SEB

We also cultured T cells with endothelial cells preincubated with SEB. In this subset of experiments, the endothelial cells were incubated with SEB (1·0 μg/ml) for 30 min at 37°, followed by washing three times with PBS containing 1% FBS. Purified spleen T cells were plated onto the endothelial cells in complete medium for 2 hr at 37° in 5% CO$_2$. The percentage of CD4$^+$ Vβ8.1,2$^+$ T cells adhering to endothelial cells pretreated with SEB was significantly increased to a level similar to that observed when the same cells were incubated simultaneously with SEB (34·8 ± 1·6%, P < 0·01; Fig. 4).
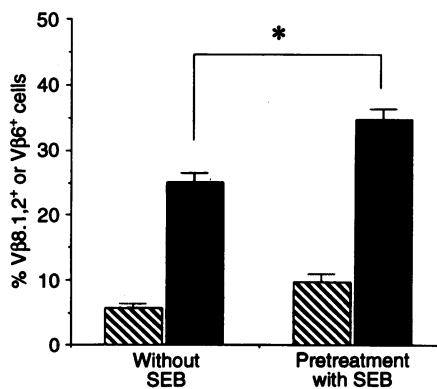

Figure 4. The percentage of adherent CD4$^+$ Vβ8.1,2$^+$ or CD4$^+$ Vβ6$^+$ cells among adherent CD4$^+$ cells: effect of pretreatment of endothelial cells with or without SEB. Endothelial cells were cultured with IFN-γ for 3 days, and pretreatment of endothelial cells with SEB was performed for 30 min followed by washing three times with PBS. Hatched bars, Vβ6$^+$ cells; closed bars, Vβ8.1,2$^+$ cells. The data are representative of two experiments. *P < 0·01.
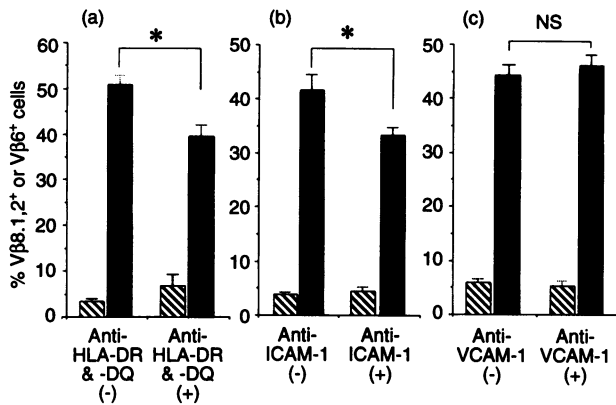
Figure 5. The percentage of CD4$^+$ Vβ8.1,2$^+$ or CD4$^+$ Vβ6$^+$ cells among adherent CD4$^+$ cells: effect of anti-HLA-DR and -DQ mAb, anti-ICAM-1 mAb, and anti-VCAM-1 mAb mAb. (a) With (left) or without (right) anti-HLA-DR and -DQ mAb (5 μg/ml). (b) With (left) or without (right) anti-ICAM-1 mAb (5 μg/ml). (c) With (left) or without (right) anti-VCAM-1 mAb (5 μg/ml). Hatched bars, Vβ6$^+$ cells; closed bars, Vβ8.1,2$^+$ cells. Monoclonal antibodies were added to the culture medium 10 min before the adherent assay. The data are representative of two experiments. *P < 0·01; NS, not significant.

Inhibition of the increased TCR Vβ-restricted T-cell adhesion by anti-SEB, anti-HLA-DR, anti-HLA-DQ and anti-ICAM-1 antibodies

The SEB-enhanced percentage of adherent CD4$^+$ Vβ8.1,2$^+$ T cells was completely blocked by the addition of anti-SEB antibody. However, the same antibody did not influence the percentage of adherent CD4$^+$ Vβ6$^+$ T cells (data not shown). Furthermore, the addition of anti-human HLA-DR and -DQ antibodies significantly diminished the increased percentage of adherent CD4$^+$ Vβ8.1,2$^+$ T cells from 51·0 ± 2·0 to 39·6 ± 2·3% (P < 0·01; Fig. 5a). The effect of anti-human ICAM-1 antibody was similar to that of HLA-DR and -DQ antibodies (41·8 ± 2·7% versus 33·4 ± 1·4%, respectively, P < 0·01; Fig. 5b), although anti-human VCAM-1 antibody lacked any effect (Fig. 5c).
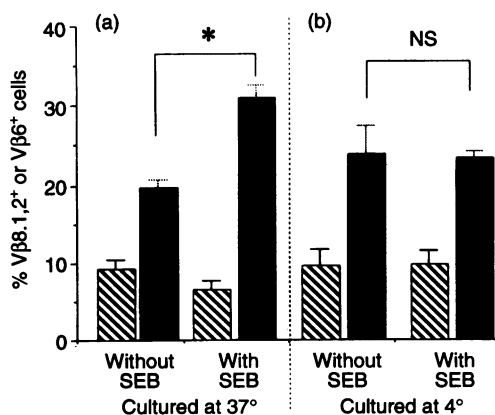

Figure 6. Percentage of CD4$^+$ Vβ8.1,2$^+$ or CD4$^+$ Vβ6$^+$ cells among adherent CD4$^+$ cells: effect of culture temperature. An adherent assay of spleen T cells to the IFN-γ-treated endothelial cells was performd at (a) 37° and (b) 4° with or without SEB. Hatched bars, Vβ6$^+$ cells; closed bars, Vβ8.1,2$^+$ cells. The data are representative of three experiments. *P < 0·01; NS, not significant.

Effect of low temperature on TCR-restricted T-cell adhesion

Co-culture of T cells with endothelial cells in the presence of SEB at 4° did not increase the percentage of adherent CD4$^+$ Vβ8.1,2$^+$ T cells (23·4 ± 0·8%; Fig. 6b). This was in contrast with the enhanced percentage of adherent cells observed at 37° (31·0 ± 1·7%; Fig. 6a).

DISCUSSION

Several reports have demonstrated that accumulated T cells in the synovial tissue or fluid in patients with RA exhibit a skewed TCR Vβ repertoire, although the predominant TCR Vβ in the lesion is still controversial.[4–10] Selective accumulation of T cells is reported in TCR Vβ–Dβ–Jβ gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of MS.[11] T cells responsive to the myelin basic protein were abundant in the CNS of EAE.[3,13–15] The present study was performed to investigate the mechanisms causing a preferential accumulation of T cells in the inflamed lesions. One possible mechanism involves proliferation of a TCR Vβ T-cell phenotype that responds to putative autoantigen or superantigen in the affected lesions. However, in the synovial tissue of RA patients only a small percentage of T cells is actively proliferating.[16,17]

Early studies demonstrated that different adhesion molecules contributed synergistically to TCR-specific interactions while the antigen-specific adhesion was maintained.[18,19] However, it is possible that adhesion molecule interactions precede TCR binding to the antigen, and that the TCR is important only in triggering T-cell effector function.[20,21] Our study has demonstrated that, in the presence of SEB, non-immunized T cells expressing a particular TCR Vβ on their surface could adhere to endothelial cells specifically with their TCR Vβ. Thus, the specific migration of T cells to the target site may occur after specific T-cell adhesion to endothelial cells.

Recently, the migration of lymphocytes from the vascular system into inflammatory sites has been demonstrated to be induced by certain cytokines, such as interleukin (IL)-1,[22] IL-2,[23] IL-3, IL-4, IL-6,[24] IL-8,[25–28] regulated on activation of normal T cells expressed and secreted (RANTES),[28,29] macrophage inflammatory protein-1α (MIP1-α) and MIP1-β.[30] Although these cytokines can induce random activation and adhesion of lymphocytes, they cannot induce migration of T cells in a TCR Vβ-specific manner. Emigration of lymphocytes from the vascular space to extravascular sites starts following a contact with the vessel wall. Then lymphocytes roll along the endothelium, and stick firmly to the endothelial cells.[31–33] The present study has only demonstrated that SEB, a superantigen, specifically augments adhesion of murine T cells bearing a particular Vβ family to human endothelial cells *in vitro*. However, if some TCR Vβ-restricted antigen-driven mechanisms exist for the selection in this cascade, the stage of selection should be in the area where the endothelial cells present the antigen to the lymphocytes.

In the present study, human but not murine MHC class II molecules, e.g. HLA-DR and -DQ, were required by specific murine T cells for the recognition of SEB, based on the following results.

(1) An SEB-induced increase in adherent CD4$^+$ Vβ8.1,2$^+$ T cells occurred when endothelial cells were pretreated with IFN-γ.

(2) Pretreatment of endothelial cells with SEB followed by a thorough washing did not lower the increase of adherent CD4$^+$ Vβ8.1,2$^+$ T cells to endothelial cells.

(3) An SEB-induced increase in adherent CD4$^+$ Vβ8.1,2$^+$ T cells was inhibited by antibodies against HLA-DR and -DQ antigens.

As MHC class II molecules, SEB and TCR are thought to form a tri-molecular complex,[34,35] a direct adhesion of CD4$^+$ Vβ8.1,2$^+$ T cells to the endothelial cells through SEB may be one of the mechanisms causing increased adhesion. However, no significant increase in CD4$^+$ Vβ8.1,2$^+$ T-cell adhesion was observed when the incubation temperature was set at 4°. The result suggests that the mechanism of increased adhesion of CD4$^+$ Vβ8.1,2$^+$ T cells by SEB is not the result of a direct binding between T cells and endothelial cells through the tri-molecular complex of MHC class II, SEB and TCR, but rather owing to adherence through newly synthesized adhesion molecules or those with conformational changes occurring after SEB stimulation. The inhibition of the SEB-induced increase in CD4$^+$ Vβ8.1,2$^+$ T-cell adhesion by anti-ICAM-1 mAb supports the latter argument. In this regard, activation of lymphocytes by TCR is known to induce a rapid increase in the strength of adhesion between LFA-1 and ICAM-1 or ICAM-2 in a metabolic energy-dependent way.[36] Our results demonstrating an inhibitory effect of low temperature on the SEB-induced increase in adhesion of CD4$^+$ Vβ8.1,2$^+$ T cells suggest that the SEB-induced increase may occur through LFA-1 and ICAM interaction. Thus, enhanced adhesion of these T cells may result from a conformational change in LFA-1 on the surface of SEB-activated Vβ8.1,2$^+$ T cells.[36] Several sources of evidence support the idea that interaction of murine LFA-1 and human ICAM-1 may occur. The sequence of murine LFA-1α and LFA-1β is highly homologous to its human counterpart,[37,38] and inhibition of human T-lymphocyte sequestration by anti-LFA-1 in xenotransplantation indicates that the interaction between LFA-1 and ICAM occurs even in the xenogenic response.[39] In contrast, Johnston et al.[40] reported conflicting data that murine LFA-1 does not bind to human ICAM-1, while murine ICAM-1 does bind to human LFA-1. The present study investigated murine T-cell adhesion to human endothelial cells, which do not express LFA-1 on their surface. In fact, the percentage of adherent murine T cells to human endothelial cells in the present study was 1–2% (data not shown), which is equivalent to the data of murine lymphocyte adhesion to human ICAM-1 reported by Johnston et al.[40] Therefore, the binding of murine LFA-1 to human ICAM-1 may be weak compared with that of human LFA-1 to murine ICAM-1, and the murine T-cell adhesion to human endothelial cells which lacked LFA-1 on their surface in the present study may depend on the binding of murine LFA-1 to human ICAM-1. As a result, the murine T-cell adhesion to human endothelial cells augmented by SEB might be inhibited by anti-ICAM-1 mAb.

EA.hy926 is a human endothelial cell line which expresses factor VIII-related protein and ICAM-1.[41–43] In this regard, stimulation of this endothelial cell line by IFN-γ does not alter the expression of adhesion molecules;[42] however, an augmented expression of ICAM-1 as well as HLA-DR, but not VCAM-1, was observed (data not shown) in accordance with the results of inhibition of the SEB-induced increased adhesion of Vβ8.1,2$^+$ T cells by each mAb.

In the present study, a large proportion of increased adherent T cells was CD4$^+$ T cells, whereas SEB is known to stimulate the proliferation of CD4$^+$ as well as CD8$^+$ T cells.[44] The mechanism underlying the difference in proliferation and adherence between the two T-cell types is not clear. However, the CD4 molecule may play an important role in the SEB-induced T-cell proliferation[45] and in the SEB-induced increase of T-cell adhesion through a direct binding to MHC class II molecules.[46]

ACKNOWLEDGMENTS

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