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DIFFERENCE IN HUMAN AND MURINE T CELL RESPONSIVENESS TO STAPHYLOCOCCAL ENTEROTOXINS IS DETERMINED BY ACTIVITIES OF MHC CLASS II-POSITIVE CELLS

Mizuho NISHIKAWA[1)4)], Junji YAGI[1)], Xiao-Jie YAN[1)], Yoko OSHIMI[2)], Shun-ichi MIYAZAKI[2)] and Takehiko UCHIYAMA[1)3)5)]

[1)]Department of Microbiology and Immunology, [2)]Department of Physiology, [3)]Department of Infectious Diseases, [4)]Department of Gastroenterology, [5)]Institute of Laboratory Animals, Tokyo Women's Medical College

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Bacterial superantigens (SAGs) bind to major histocompatibility complex (MHC) class II molecules on accessory cells (AC) and stimulate T cells upon interaction with the Vβ portion of the T cell receptor (TCR). We have recently shown that bacterial SAGs produced by *Staphylococcus aureus*, Staphylococcal enterotoxin A (SEA), SEB, SEC, and toxic shock syndrome toxin-1 (TSST-1) can all stimulate human peripheral blood mononuclear cells (PBMC) at very minute doses of antigens ($\geq 10^{-4}$ ng/ml). By contrast, when murine peripheral lymphocytes are used, SEs and TSST-1 have been segregated into two groups according to potency. SEA and TSST-1 are equally strong stimulators of murine peripheral lymphocytes ($\geq 10^{-4}$ ng/ml), whereas the responses of murine peripheral lymphocytes to SEB and SEC require 10^3～10^4-fold greater doses. However, it is still unclear whether this difference between the murine and human responses to SEB and SEC is due to a difference in T cell responsiveness or a difference in the activity of AC. In this study, the response of identical murine T cell preparations to SEB showed a preference for human AC over murine AC. Thus, the results indicated that the difference in response to SEB is primarily due to a difference in the activity of AC. Futhermore, the Ca^{++} concentration of murine T cells responding to SEB presented by human AC was higher than that of those responding to SEB presented by murine AC, indicating that different accessory activity influences T cell activation from the early phase of signal transduction.

Introduction

Superantigens (SAGs) activate a large proportion of T cells upon interaction with the Vβ portion of the T cell receptor (TCR) in direct association with major histocompatibility complex (MHC) class II molecules on accessory cells (AC)[1)~5)]. SAGs are classified into two groups. One group, called viral SAGs, is the product of open reading frames of a 3′-long terminal repeat in the mouse mammary tumor virus[4)]. The other group, called bacterial SAGs, contains staphylococcal products [staphylococcal enterotoxin A (SEA), SEB, SEC, SED and SEE, and toxic shock syndrome toxin-1 (TSST-1)], streptococcal pyrogenic exotoxins and a yersinial product [*Yersinia pseudotuberculosis*-derived mitogen (YPM)][1)2)5)]. These SAGs have been implicated in the pathogenesis of acute and systemic diseases such as toxic shock syn-

drome and scarlet fever. We and others consider that potent T cell stimulatory activities of SAGs are primarily involved in these abnormal disease reactions[5)~8)].

Bacterial SAGs have been shown to induce marked activation of both murine and human T cells. However, if one examines the relative potencies among bacterial SAGs, some bacterial SAGs show different potencies in the activation of murine and human T cells. In this context, we have shown that SEA, SEB, SEC and TSST-1 induce equally marked activation of human peripheral blood mononuclear cells (PBMC), whereas SEs and TSST-1 are classified into two groups in terms of their potency to activate murine peripheral lymphocytes[9)]. SEA and TSST-1 can activate murine peripheral lymphocytes from similar or slightly higher doses in comparison with those for human PBMC. However, SEB and SEC require 10^3 $\sim 10^4$ higher doses than SEA and TSST-1 for the activation of murine peripheral lymphocytes. This observation led us to further examine whether the different strengths of T cell response to SEB and SEC between murine and human cells are primarily determined by T cell responsiveness or the activity of AC. In this study, we compared the response of T cells to SEA and SEB in the presence of human MHC class II molecule-positive cells with that in the presence of murine MHC class II molecule-positive cells, and we found that the difference in strength between human and murine peripheral lymphocyte responses observed in response to SEB is due to the difference in the activity of AC.

Materials and Methods

Animals

$B6C3F_1$ mice were purchased from Japan S.L. C., Inc., Hamamatsu, Japan. C57BL/6 mice were bred in our own colonies at the Department of Microbiology and Immunology, Tokyo Women's Medical College, Tokyo, Japan.

Monoclonal antibodies and reagents

mAbs to I-A^b (28-16-8S), I-E (14.4.4S) and CD8 (83.12.5) were described previously[10)]. mAb to B cell antigen (LR-1) and Thy 1.2 antigen (HO13) were purchased from Serotec Ltd. (Oxford, England). A panel of anti-Vβ antibodies [anti-Vβ2; B20.6[11)], anti-Vβ3; KJ25, anti-Vβ4; KT4-10[12)], anti-Vβ5; MR9-4[13)], anti-Vβ6; RR4-7, anti-Vβ7; TR310[14)], anti-Vβ8; F23.1, anti-Vβ9; MR10-2[15)], anti-Vβ10; KT10b-2[16)], anti-Vβ11; RR3-15, anti-Vβ12; KT12-15 and anti-Vβ14; 14.2[17)]] was used. KJ25, RR4-7, F23.1 and RR3-15 were described previously[18)]. B20.6, KT4-10, KT10b-2, KT12-15 and 14.2 were obtained from Dr. K. Tomonari (Fukui Medical School, Fukui, Japan). MR9-4 and MR10-2 were obtained from Dr. O. Kanagawa (Washington University, St. Louis, MO). TR310 was the gift of Dr. C.A. Janeway, Jr. (Yale University, New Haven, CT). SEA and SEB were purchased from Toxin Tec., Inc. (Sarasota, FL).

L cell transfectants

L cells transfected with I-A^b genes (FT6.2), DR4 and Dw13 genes (8124) and control L cells (Lmc and 8400) were described previously[19)20)]. These L cell transfectants were used as AC after being inactivated by mitomycin C and irradiated with 3500 rad.

Preparation of murine cells

Single spleen cell suspensions were prepared in Hanks' solution with 2% FCS. Erythrocytes were lysed with Tris-NH_4Cl. Spleen AC were obtained by treatment of spleen cells with anti-Thy 1.2 and guinea pig complement, and were inactivated by mitomycin C. T cells were obtained from spleen cells by passing them through a nylon wool column or taking non-adherent cells to goat anti-mouse Igs-coated plates (1001 Falcon, Becton Dickinson Labware, Oxnard, CA). $CD4^+$ T cells were purified by further treatment of T cells with a mixture of anti-I-A, anti-I-E, anti-CD8 and anti-B cell antigen antibodies and guinea pig complement as described previously[10)]. Enterotoxin-induced T cell blasts were obtained by stimulating $CD4^+$ T cells with SEA (100 ng/ml) or SEB (10 μg/ml) in the presence of spleen AC. After 3 days of

culture, blast cells were collected by applying them to a discontinuous Percoll (Pharmacia, Uppsala, Sweden) gradient (densities of 1.060 and 1.075). Recovered blast cells fractionated at the interfaces of 1.060 and 1.075 were expanded in the presence of murine rIL-2 (100 U/ml) for 2 days and purified by Percoll gradient before use.

Preparation of human cells

PBMC were obtained from the peripheral blood of healthy donors by using a Ficoll-Conray density gradient. Human AC were obtained from PBMC by the S-2-aminoethylisothiouronium-treated SRBC rosette method as the nonrosette-forming cell fraction, as described previously[19]. Two rounds of this procedure were performed ($CD3^+$ cells, <1%). T cells were purified by applying the rosette-forming cells to nylon wool columns ($CD3^+$ cells, >98%).

Culture medium

RPMI 1640 containing 100 μg/ml of streptomycin, 100 U/ml of penicillin, 10% FCS and 5×10^{-5} M 2-ME was used for the culture of cells.

Assay for IL-2 production

Various numbers of whole spleen cells, PBMC or T cells plus AC were cultured as indicated in each experiment. Supernatants were obtained at appropriate periods of culture. IL-2 production in the culture supernatants was assayed by the method described previously[21].

Analysis of $[Ca^{++}]_i$ in enterotoxin-reactive murine T cell blasts

$[Ca^{++}]_i$ was analyzed as described previously[22]. SEA- or SEB-induced murine T cell blasts were loaded with the Ca^{++}-sensitive fluorescent dye fura 2 by incubation with its acetoxymethyl derivative, fura 2 AM (Molecular Probes, Eugene, OR), at a concentration of 5 μM at 37°C for 30 min. After SEB or SEA was added to the 1-to-1 mixture of fura 2-loaded blast cells and human or murine MHC class II-positive L cells, the Ca^{++} concentration in a responding blast cell was measured every 30s for 20 min. For the activation of fura 2 fluorescence, fura 2-loaded blast cells were irradiated by ultraviolet light of 340 nm in wavelength (UV340) for 0.5s, and 1s later, by UV380 for 0.5 s. Paired UV340 and UV380 irradiation was applied every 30s for 20 min. To measure the Ca^{++} concentration in a responding cell, the ratio of linear fluorescence at UV340 to that at UV380 for each pair of UV340 and UV380 was processed with an image processor (Argus 100, Hamamatsu Photonics, Hamamatsu, Japan).

Assay for Vβ repertoire of T cells responding to SEB

SEB-induced murine T cell blasts were obtained by stimulating 1.5×10^6 C57BL/6 $CD4^+$ T cells with SEB (10 μg/ml) in the presence of 1.5×10^6 normal murine AC or 1.5×10^6 normal human AC in 24-well culture plates. After 3 days of culture, blast cells were collected by layering onto a Percoll gradient, and then expanded in the presence of IL-2 for 2 days. Recovered blast cells, or C57BL/6 $CD4^+$ T cells before stimulation with SEB, were incubated with anti-Vβ antibodies followed by FITC-goat $F(ab')_2$ anti-mouse IgG or FITC-goat anti-rat IgG. Samples of 10,000 viable cells were analyzed on an Epics CS flow cytometer. The percent of T cells expressing different Vβ-encoded TCR was calculated by subtracting background staining in the absence of first antibodies and corrected by the percent of T cells (>90%) as determined by staining with FITC-anti-CD3.

Results

Marked difference between murine and human peripheral lymphocytes is observed in the response to SEs

We have previously shown that a panel of SEs and TSST-1 could segregate into two groups according to the potency of the murine T cell response, as stated in the **Introduction**. On the other hand, there was no marked difference in potency among toxins in the human response[9]. In this study, murine spleen cells or human PBMC were stimulated with a wide dose range of SEA and SEB, and levels of response were determined by the amount of IL-

2 produced by the T cells. As shown in Fig. 1A, SEA could stimulate murine spleen cells and human PBMC to produce IL-2 at doses of 10^{-3} and 10^{-4} ng/ml, respectively. However, SEB showed much lower potency in the activation of murine spleen cells than that in the activation of human PBMC (Fig. 1B). Murine spleen cells required dose of SEB (10^{1} ng/ml) about 10^{4}-fold greater than that needed for the response to SEA. PBMC appeared to require similar doses of SEA and SEB in responses ($\geq 10^{-4}$ ng/ml). Thus, the results clearly indicate that SEA is far more potent mitogen than SEB in the activation of murine peripheral lymphocytes, while SEB and SEA are highly potent in the activation of human peripheral lymphocytes.

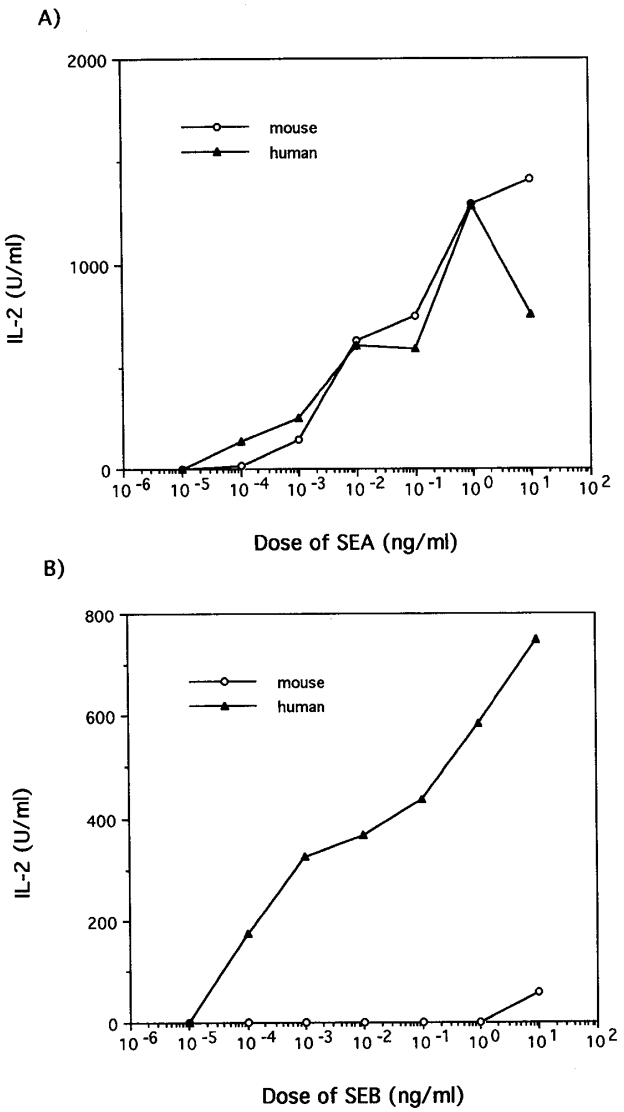

Fig. 1 Doses of SEA and SEB required for the response of murine spleen cells and human PBMC to produce IL-2

3×10^{5} C57BL/6 whole spleen cells (—○—) or 3×10^{5} human PBMC (—▲—) were cultured in 0.2-ml volumes in 96-well flat-bottom microtiter plates in the presence of titrated amounts of SEA (A) and SEB (B). After 72 h for spleen cells or 24 h for PBMC, IL-2 production in the culture supernatant was determined by the proliferation of CTLL-2.

Different potencies of SEs in the murine and human T cell responses are determined by AC, and not by T cells

To gain insight into the nature of different potency of SEB in the activation of murine and human T cells, we next examined whether they are due to a difference in T cell responsiveness or the effects of AC. To determine this, identical murine T cell preparations were stimulated in the presence of either murine T-depleted spleen cells or human T-depleted PBMC with a wide dose range of SEA and SEB. Murine T cells did not produce IL-2 without SEs in the presence of human AC, indicating that the xenogenic effect was negligible (data not shown). In this system, murine T cells were stimulated with 10^{-1} ng/ml or more of SEA to produce IL-2 in the presence of murine AC and human AC (Fig. 2A). Whereas, identical T cell preparations required about 10^{2}-fold more SEB (10^{2} ng/ml) to produce IL-2 in the presence of murine AC than that (10^{0} ng/ml) in the presence of human AC (Fig. 2B). Thus, different potencies of SEB were observed in the co-culture of mouse T cells with murine AC or human AC, suggesting that the difference in potency of SEB in the activation of murine and human T cells is primarily determined by AC and not by T cells.

The response of SE-induced T blasts also shows a preference for human AC

To further investigate the potencies of SEA and SEB in the activation of murine T cells as opposed to human T cells, we used SEA- or SEB-induced T blasts as responders that have a limited Vβ repertoire in their TCR. SEA- or SEB-induced mouse T blasts were stimulated in

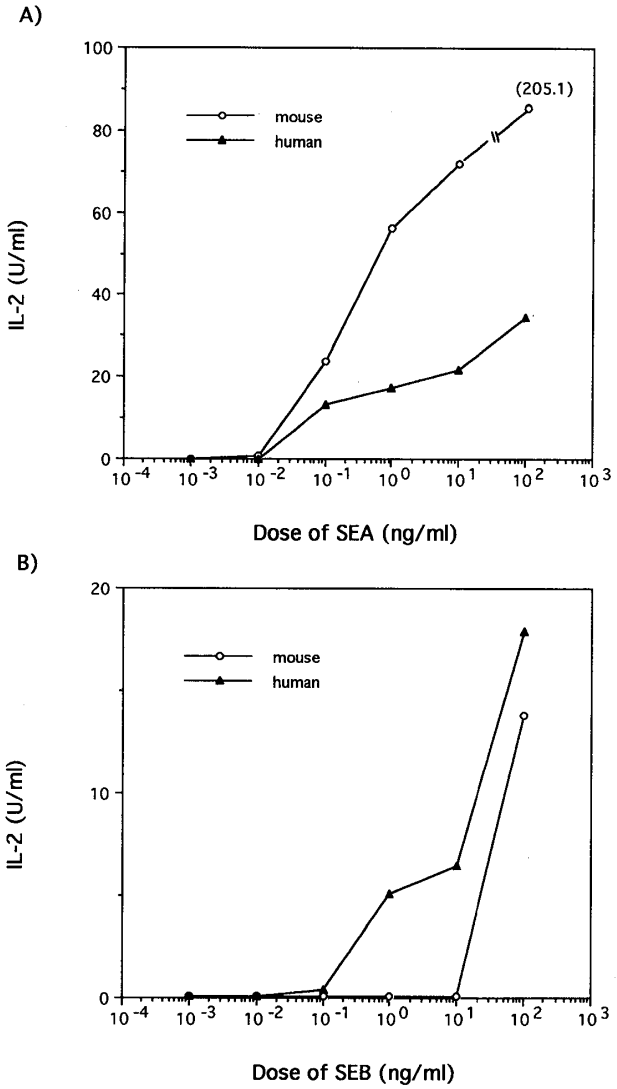

Fig. 2 Activities of normal murine AC and normal human AC to support the murine T cell response to SEA and SEB

C57BL/6 $CD4^+$ T cells were obtained from spleen cells by passage through a nylon wool column and treatment with a mixture of anti-I-A^b, anti-CD8 and anti-B cell antigen antibodies and guinea pig complement, and were used as responder cells. Murine AC were obtained by treatment of C57BL/6 spleen cells with anti-Thy1.2 and guinea pig complement. Human AC were obtained by two rounds of the S-2-aminoethylisothiouronium-treated SRBC rosette method as a nonrosette-forming PBMC fraction. Murine and human AC were inactivated by mitomycin C. 1×10^5 C57BL/6 $CD4^+$ T cells were cultured with 1×10^5 normal murine AC (—○—) or 1×10^5 normal human AC (—▲—) in 0.2-ml volumes in 96-well flat-bottom microtiter plates in the presence of titrated amounts of SEA (A) or SEB (B) for 72 h. IL-2 production in the culture supernatant was determined as in Fig. 1.

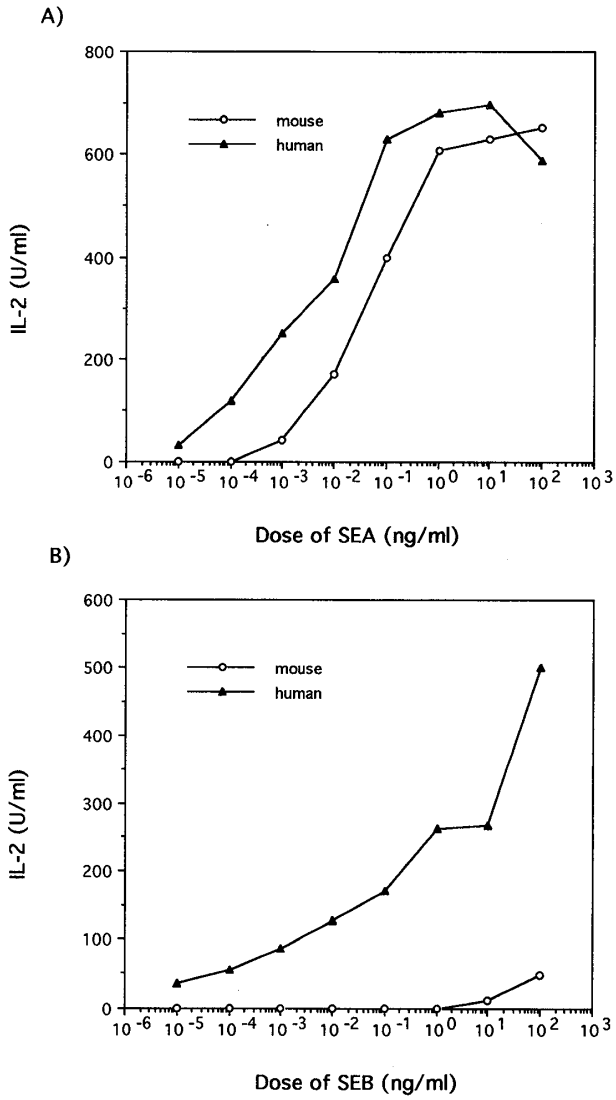

Fig. 3 Activities of normal murine AC and normal human AC to support the murine T cell blast response to SEA and SEB

SEA- or SEB-induced murine T cell blasts were obtained by stimulating 1.5×10^6 C57BL/6 $CD4^+$ T cells with SEA (100 ng/ml) or 1.5×10^6 B6C3 F1 $CD4^+$ T cells with SEB (10 μg/ml) in the presence of 1.5×10^6 syngeneic murine AC in 1.5-ml volumes in 24-well culture plates. After 3 days of culture, blast cells were collected by layering onto a discontinuous Percoll gradient. Recovered blast cells were expanded in the presence of 100 U/ml of mouse rIL-2 for 2 days, and purified using a Percoll gradient. 1×10^5 T cell blasts were cultured with 1×10^5 syngeneic normal murine AC (—○—) or 1×10^5 normal human AC (—▲—) in 0.5-ml volumes in 48-well culture plates in the presence of titrated amounts of SEA (A) or SEB (B). After 20 h of culture, IL-2 production in the culture supernatant was determined as in Fig. 1.

the presence of either normal murine AC or normal human AC with SEA or SEB. As shown in Fig. 3A, SEA-induced T blasts responded to SEA from 10^{-3} ng/ml in the presence of murine AC and from 10^{-5} ng/ml in the presence of human AC. SEB-induced T blasts required about 10^6-fold greater dose of SEB (10^1 ng/ml) to produce IL-2 in the presence of murine AC than that in the presence of human AC (Fig. 3 B). These dose-response curves are similar to those of whole spleen cells and human PBMC as shown in Fig. 1, indicating that the different responses to SEB of murine and human T cells are determined at the level of AC.

In order to exclude the effects of molecules other than MHC class II on the different potencies of SEB in the activation of murine and human T cells, similar experiments were carried out using L cells transfected with MHC class II genes. SEA-induced mouse T blasts were stimulated with SEA to produce IL-2 in the presence of I-A$^+$ L cells and DR$^+$ L cells at and above 10^{-2} and 10^{-4} ng/ml, respectively (Fig. 4A). SEB-induced blasts were stimulated from about a 10^5-fold greater dose of SEB (10^1 ng/ml) to produce IL-2 in the presence of I-A$^+$ L cells than that in the presence of DR$^+$ L cells (Fig. 4B). Thus, these results indicate that the presentation of SEB by MHC class II molecules primarily determines the potency of SEB in the activation of T cells.

Different potencies of SEB in the activation of murine and human cells are observed in the triggering of early signal transduction, and are determined by AC

It has been shown that bacterial SAGs induce the elevation of $[Ca^{++}]_i$ in the early signal transduction that occurs in T cells after their interaction with TCR[23]. Therefore, we next examined whether the different SEB-presenting activities of murine AC and human AC affect the level of $[Ca^{++}]_i$ elevation in T cells. To determine this, mouse SE-induced T cell blasts loaded with fura 2 in the presence of either DR$^+$ L cells or I-A$^+$ L cells were stimulated with several concentrations of SEA and SEB. SEA

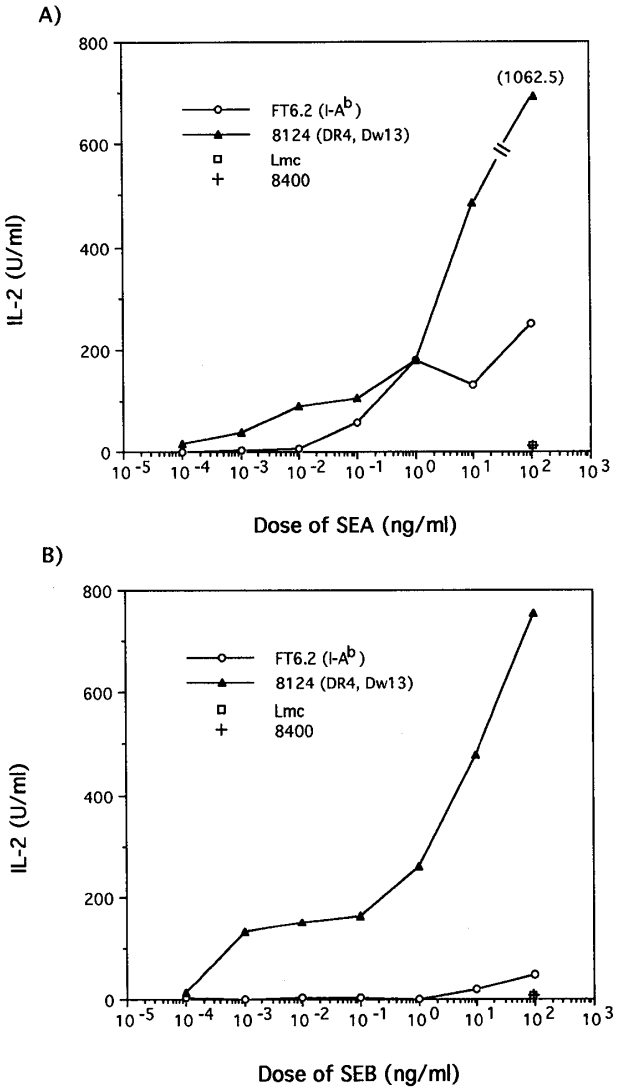

Fig. 4 Activities of murine MHC class II-positive L cell transfectants and human MHC class II-positive L cell transfectants to support the murine T blast response to SEA and SEB
2×10^5 SEA- or SEB-induced T cell blasts obtained as in Fig. 3 were cultured with 2×10^4 murine MHC class II-positive L cell transfectants [FT6.2 (I-A^b)] (—○—) or 2×10^4 human MHC class II-positive L cell transfectants [8124 (DR4, Dw13)] (—▲—) in 1.5-ml volumes in 24-well culture plates in the presence of titrated amounts of SEA (A) or SEB (B). Lmc (□) and 8400 (+) were used as control L cells. After 20 h of culture, IL-2 production in the culture supernatant was determined as in Fig. 1.

presented by DR$^+$ L cells and I-A$^+$ L cells did not induce different $[Ca^{++}]_i$ in SEA-induced T cell blasts (Fig. 5A). At 10 μg/ml, SEB induced similar levels of $[Ca^{++}]_i$ elevation in SEB-

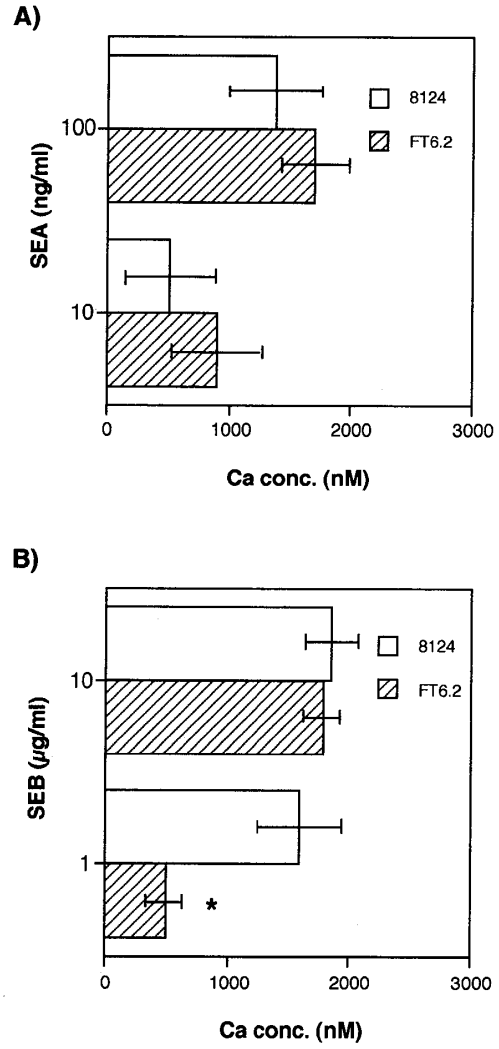

Fig. 5 Different potencies of SEB in the activation of murine and human cells are observed in the triggering of early signal transduction, and are determined by AC

SEA (A) or SEB (B) was added to the 1-to-1 mixture of fura 2-loaded SEA- or SEB-induced T blasts obtained as in Fig. 3 and human MHC class II-positive L cells (8124) or murine MHC class II-positive L cells (FT6.2). Ca^{++} concentrations in responding T blasts were obtained every 30s for 20 min. Results are shown as means ± S.D. of maximum concentrations of seven individual responding cells. The significance of differences was analyzed by Student's *t*-test, and indicated as *$p<0.001$.

induced T blasts in the presence of DR^+ L cells and $I\text{-}A^+$ L cells. However, at 1 μg/ml, SEB induced significantly weaker elevation of $[Ca^{++}]_i$ in SEB-induced T blasts in the presence of $I\text{-}A^+$ L cells than in the presence of DR^+ L cells (Fig. 5B). Thus, the preference for DR molecules on AC in the response to SEB is observed in the triggering of early transduction of T cell activation by SEB.

Vβ repertoires of T cells responding to SEB in the presence of murine AC and human AC are similar

Responding T cells also may contribute to the different potencies of SEB. SEB presented by human AC could have a different tertiary structure from SEB presented by murne AC, resulting in different Vβ repertoires in responding T cells. To examine this possibility, mouse normal $CD4^+$ T cells were stimulated with 10 μg/ml SEB in the presence of human normal AC or murine normal AC. Recovered blast cells were expanded in the presence of rIL-2 and examined for TCR Vβ expression (Table). SEB-induced T blasts bearing Vβ3 and Vβ8 gene products on their TCR were clearly enriched in the presence of murine AC, as reported previously[2]. SEB-induced T blasts in the presence of human AC also showed increased proportions of Vβ3- and Vβ8-bearing T cells. In contrast, the proportions of other Vβ-bearing T cells showed relative decreases during the response or increased only marginally. Thus, the Vβ repertoire of mouse T cells responding to SEB presented by human MHC class II molecules is similar to that to SEB presented by murine MHC class II molecules. This result suggests that the different potencies of SEB in the activation of murine T cells and human T cells are not due to different Vβ repertoires in the responding T cells.

Discussion

As our earlier studies have shown, SEA was potent mitogen to murine spleen cells and human PBMC, eliciting responses at as low as 10^{-3} and 10^{-4} ng/ml, respectively. However, 10^5 ~10^6-fold higher concentration of SEB was required for the activation of murine spleen cells than that required for the activation of human PBMC. Thus, SEA and SEB can be classified into different phenotypes according

Table Vβ repertoire of murine T cells responding to SEB in the presence of murine AC and human AC

	Percent of T cells expressing												
	Vβ2	Vβ3	Vβ4	Vβ5	Vβ6	Vβ7	Vβ8	Vβ9	Vβ10	Vβ11	Vβ12	Vβ14	Total
Normal CD 4+ T cells	5.4	3.8	6.4	3.1	8.6	1.6	20.0	0.7	3.7	5.0	3.3	5.6	67.2
SEB-induced blasts in the presence of murine AC	N.D.	13.5	N.D.	4.0	1.5	N.D.	30.5	0.9	N.D.	2.0	N.D.	N.D.	—
SEB-induced blasts in the presence of human AC	3.7	8.2	3.7	2.1	3.8	2.0	36.9	1.0	1.5	3.8	0.3	2.6	69.6

C57BL/6 CD4+ T cells and murine or human AC were obtained as in Fig. 2. SEB-induced murine T blasts were obtained by stimulating 1.5×10^6 C57BL/6 CD4+ T cells with SEB (10 μg/ml) in the presence of 1.5×10^6 syngeneic normal murine AC or 1.5×10^6 human AC in 1.5-ml volumes in 24-well culture plates. Blast cells were collected and expanded as in Fig. 3. Blast cells, or C57BL/6 CD4+ T cells before stimulation with SEB were incubated with anti-Vβ antibodies (anti-Vβ2; B20.6, anti-Vβ3; KJ25, anti-Vβ4; KT4-10, anti-Vβ5; MR9-4, anti-Vβ6; RR4-7, anti-Vβ7; TR310, anti-Vβ8; F23.1, anti-Vβ9; MR10-2, anti-Vβ10; KT10b-2, anti-Vβ11; RR3-15, anti-Vβ12; KT12-15, anti-Vβ14; 14.2), followed by FITC-goat $F(ab')_2$ anti-mouse IgG or FITC-goat anti-rat IgG. Samples of 10,000 viable cells were analyzed on an Epics CS flow cytometer. The percent of T cells expressing different Vβ-encoded TCR was calculated by the subtraction of background staining in the absence of first antibodies and corrected by the percent of T cells (>90%) as determined by staining with FITC-anti-CD3.
N.D.: not done.

to their ability to activate murine T cells. In terms of this classification, SEE and TSST-1 belong to the SEA phenotype, whereas SEC belongs to the SEB phenotype[9].

In this study, a similar dose-response pattern was observed in co-cultures of murine T cells with murine normal AC or human normal AC in the presence of a wide dose range of SEA and SEB. SEB presented by human AC was a far more potent stimulator of murine T cells than that presented by murine AC, whereas SEA presented by human AC and murine AC gave rise to equally strong activation of murine T cells. Similar results were obtained by using L cell transfectants with MHC class II genes as AC. Therefore, it is suggested that the different potencies of SEB in the activation of murine and human lymphocytes are primarily due to the different SEB-presenting activities of murine and human MHC class II molecules. Equally, similar potencies of SEA in the activation of murine and human lymphocytes suggest similar SEA-presenting activities of murine and human MHC class II molecules. Strongly supporting this notion, the responses of murine T cell blasts induced by SEB in the presence of murine normal AC, which are therefore responsive to SEB presented by murine MHC class II molecules, also showed the preference for human AC but not murine AC in their response to SEB. In addition, as the Ca^{++} concentration of murine T cells responding to SEB in the presence of murine AC was lower than that in the presence of human AC, the lower SEB-presenting activity of murine MHC class II molecules affects T cell activation from the early phase of signal transduction and leads to the production of a lesser amount of IL-2.

Recent study has indicated that some murine T clones respond to SE which is not associated with their Vβ in the presence of human AC[24]. However, the Vβ repertoire of murine T cells responding to SEB in the presence of human AC was comparable with that in the presence of murine AC in this study. Thus, the different SEB-presenting activities of murine and human AC are not likely to generate different repertoires of murine T cells responding to SEB in bulk culture.

What then could account for the preference for human AC in the response to SEB? A simple explanation would be that SEB has a higher affinity for human MHC class II molecules than murine MHC class II molecules, resulting in a

greater amount of SEB for presentation to T cells. However, it is not well established in the T cell response to bacterial SAGs that the affinity of SE for MHC class II molecules can correlate with potency in the activation of T cells, as discussed previously[5]. Direct high-affinity binding of SEA to HLA-DR and HLA-DQ has been shown by several laboratories[25)~27)], whereas the binding of SEA to murine MHC class II molecules shows much lower affinity than that for human MHC class II molecules[24]. Despite this fact, the results of this study clearly indicated that murine T cell response to SEA in the presence of human AC is almost equivalent to that in the presence of murine AC. Furthermore, though both anti-I-A and anti-I-E antibodies have shown equivalent inhibition of the direct binding of SEB to mouse B cell lymphoma cells, the response of T cells to SEB has shown a preference for presentation by the I-E molecule over the I-A molecule[28]. Therefore, one might find it difficult to account for the preference for human AC by the different affinities of SEB for different MHC class II molecules.

Bacterial SAGs have not been shown to bind directly to TCR[29], and immobilized ones could not stimulate T cells. In addition, soluble β chains of TCR can interact with SEA only when they are complexed with MHC class II molecules[30]. These resuls suggest at least two possible mechanisms by which bacterial SAGs stimulate T cells. First, T cells may recognize an altered conformation of SEB after binding to MHC class II molecules. The results of this study may be explained by stating that human MHC class II molecules generate sites on SEB that bind more effectively to the Vβ-encoded portion of TCR than murine MHC class II molecules. However, X-ray crystallography has recently shown that no large conformational change occurs in SEB with the binding to MHC class II molecule[31], suggesting the rigid molecular structure of SEB. Therefore, this possibility seems less likely. Second, the response of T cells to SEs might require simultaneous interaction of TCR with SE and MHC class II molecules. As Janeway et al originally proposed, SE complexed with MHC class II molecules will align the TCR over MHC class II molecules[32]. If the alignment of TCR over MHC class II molecules is done properly, T cells will be activated through the interaction between TCR and MHC class II molecules. Recent study by X-ray crystallography has shown that SEB complexed with DR1 molecules orients TCR in close proximity to the peptide-binding sites on MHC class II molecules[31]. Thus, the trimolecular complex of TCR, MHC class II molecule and SE might be more properly positioned by the binding of SEB to human MHC class II molecules than mouse MHC class II molecules, suggesting that SEB has evolved to do so. This might explain the contribution of the Vα portion or CDR1 β region of TCR to the response to bacterial SAGs[33)~35)]. Further analyses of structures such as SEB complexed with murine MHC class II molecules are necessary to determine whether this is the case.

Bacterial SAGs have been found to cause various diseases. SEs and streptococcal pyrogenic exotoxins are pathogenic agents of toxic shock syndrome and staphylococcal scalded syndrome, and of toxic shock syndrome and scarlet fever, respectively[5]. In mice, the administration of SEB causes weight loss and immunosuppression[8]. Since these effects are T cell dependent, an excess amount of lymphokines secreted by a large number of SAG-reactive T cells seems to be responsible for the pathogenesis, as proposed previously by us[6]. Indeed, anti-TNF antibodies abolished the induction of lethal shock triggered by SEB[36]. Thus, it is most likely that an abnormally strong T-cell reaction is directly involved in the pathogenesis of some bacterial infections. The results of this study indicate that different potencies of SEs in the activation of murine and human lymphocytes are due to the different SE-presenting activities of murine and human MHC class II molecules and not to a difference in T-cell responsiveness. It is therefore suggest-

ed that the presentation of bacterial SAGs by MHC class II molecules plays a central role in bacterial infection primarily by determining the extent of T-cell activation which leads to the abnormal disease reaction.

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細菌性スーパー抗原に対するヒトおよびマウスT細胞の反応性の差異の解析

東京女子医科大学 [1]微生物学免疫学，[2]第二生理学，
[3]感染対策科，[4]消化器内科学，[5]実験動物中央施設

西川 瑞穂[1)4)]・八木 淳二[1)]・厳 小傑[1)]
押味 蓉子[2)]・宮崎 俊一[2)]・内山 竹彦[1)3)5)]

スーパー抗原は通常のタンパク抗原とは異なり，アクセサリー細胞（A細胞）上のMHCクラスII分子に直接結合し，特定のT細胞レセプターβ鎖V領域（Vβ）を表現する大きなT細胞レパートリーと反応する．したがって，細菌性スーパー抗原は，ヒトおよびマウス末梢リンパ球に同様な強い活性化を惹き起こす．しかし，ある種の細菌性スーパー抗原staphylococcal enterotoxin B (SEB), SEC等では，マウス末梢リンパ球は，ヒト末梢リンパ球と比較して著しく低応答性である．この反応性の差が，T細胞自体の反応性の差によるものか，A細胞のアクセサリー活性の差によるものかは未だ明らかではない．細菌性スーパー抗原によるT細胞反応の強さを決定する因子を解明するため，同一T細胞を用いて，ヒトおよびマウスMHCクラスII分子陽性細胞の存在下でSEAおよびSEBに対する反応性を比較，検討した．その結果，SEBに対するヒトとマウス末梢リンパ球の反応性の差は，A細胞のアクセサリー活性の差に基づくことが示唆された．また，アクセサリー活性の差は，反応T細胞内Ca^{++}濃度にも差を惹き起こすことから，T細胞活性化の初期から影響を与えているものと考えられた．細菌性スーパー抗原による生体異常反応は，異常に強いT細胞反応に起因すると考えられている．したがって，T細胞反応の強さがA細胞のレベルで決定することが明らかとなったことは，細菌感染症の制御の上で有用であると思われる．