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Superantigenic Stimulation of Bovine T Cells by *Streptococcus dysgalactiae*-Derived Mitogen (SDM)

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Streptococcus dysgalactiae-derived mitogen (SDM) was recently identified from a bovine isolate of *S. dysgalactiae*. A comparison of its effects on human and bovine PBMC revealed that it acts as a superantigen on both species, stimulating the T cell fraction of PBMC to proliferate. It selectively targets human Vβ1 and Vβ23 positive T cells and reactive bovine T cells express the bovine TCR-Vβ chain most similar to these human targets, VβBTB18. The superantigen SpeC, which is a product of the predominantly human pathogen *Streptococcus pyogenes*, also activates corresponding T cells in human and bovine systems. SDM however, has a much more potent effect on bovine cells than human cells and is more likely to have a pathogenic role in *S. dysgalactiae* infections of cows than humans.

Key words: superantigen, bovine, T cell, streptococcus

Introduction

Superantigens (SAGs) differ from conventional T cell-stimulating antigens in several respects. They bind directly to major histocompatibility class II (MHC II) molecules expressed on accessory cells (AC) and selectively activate virtually all T cells bearing particular T cell receptor (TCR) beta chain variable (Vβ) elements upon recognition of the SAG/MHC class II complex. Conventional antigens, on the other hand, bind to MHC class I or II molecules after being processed into small sized peptides and activate a limited number of T cell clones[1)2)]. Several species of bacteria that cause disease in humans are known to produce SAGs. Notable among them are *Staphylococcus aureus*, the source of the staphylococcal enterotoxins A to M (SEA～SEM) and of toxic shock syndrome toxin-1 (TSST-1), *Streptococcus pyogenes*, which produces streptococcal pyrogenic exotoxins A to K (SpeA～SpeK) and streptococcal mitogenic exotoxins (SMEZs) and *Yersinia pseudotuberculosis*, which produces *Y. pseudotuberculosis*-derived mitogen (YPM)[3)～6)]. The excessive amounts of pro-inflammatory cytokines produced by SAG-activated T cells play a crucial role in the development of diseases such as staphylococcal and streptococcal toxic shock syndromes (TSS and STSS), neonatal TSS-like exanthematous disease (NTED), and systemic *Y. pseudotuberculosis* disease[6)～13)].

Streptococcus dysgalactiae-derived mitogen (SDM) is an SAG recently identified in our labo-

ratories, from a bovine isolate of *S. dysgalactiae*[14]. It appears to be unique to *S. dysgalactiae* isolates from cows. Human isolates have not been shown to produce this SAG. Our previous studies showed that SDM is not as potent an activator of human T cells as other SAGs produced by *S. pyogenes*. For example, the minimum dose of SDM required for the stimulation of human T cells is more than ten times that of SpeC, which is considered pathogenic to humans. It is thus less likely to have a significant pathogenic effect on humans. However, we suspected that it could have such an effect on the cow, which is the natural host of the SDM-producing bacteria. A comparative examination of the activity of SDM on human and bovine T cells was considered necessary for a fuller understanding of the pathogenic potential of SDM in *S. dysgalactiae* infections.

In this study, we compared the stimulatory activity of SDM and SpeC on bovine and human T cells. We also determined the bovine TCRβ-chain elements targeted by SDM and SpeC and compared them to the TCR Vβ elements on the human T cells they activate. We found that SDM is a more potent activator of bovine T cells than SpeC and that the bovine T cells these SAGs target, express Vβ elements most similar to those found on their human targets. The possibility of SDM's involvement in disease pathogenesis in cows and humans is discussed.

Materials and Methods

Toxins, reagents and culture medium

Recombinant SDM (r-SDM) prepared as previously described[14] was used in all experiments. Briefly, r-SDM was purified from an extract of *Escherichia coli* HB101 carrying pQE30-6xH. *sdm* using Chelating Sepharose Fast Flow (Amersham Biosciences, Buckinghamshire, UK) pre-loaded with Ni^{2+}according to manufacturer's instructions. The final product was confirmed by migration as a single band on SDS-PAGE. SpeC was purchased from Toxin Technology (Sarasota, FL, USA) and Concanavalin A from Wako Pure Chemical Industries (Osaka, Japan). Anti-CD3 monoclonal antibody (mAb), OKT3, was prepared from the producing cell line (American Type Culture Collection, Rockville, MD, USA). Human r-IL2 was kindly provided by Shionogi Pharmaceuticals. RPMI 1640 supplemented with 100 μg streptomycin/ml, 100 U penicillin/ml, 10% fetal bovine serum and 5×10^{-5} M 2-mercaptoethanol was used for all lymphocyte cultures.

Preparation of bovine and human lymphoid cells

Heparinized whole blood from healthy adult cows was purchased from Tokyo Shibaura Zoki KK (Tokyo, Japan) and human blood, collected from healthy adult volunteers. Peripheral blood mononuclear cells (PMBC) were isolated by Ficoll-Conray gradient centrifugation. T cell-depleted bovine PBMC were prepared by staining whole PBMC with bovine anti-CD2, CD4 and CD8 monoclonal antibodies (Serotec, Oxford, UK) and FITC-labelled anti-mouse IgG (Zymed Laboratories, South San Francisco, CA, USA), then using an EPICS ALTRA cell sorter (Beckman Coulter Inc., Fullerton, CA, USA) to collect both stained and unstained cells. Unlabelled cells constituted the T cell-depleted population.

Lymphocyte proliferation assays

Toxin ability to induce lymphocyte proliferation was determined by a standard ^{3}H-thymidine incorporation assay[15]. PBMC (1×10^{5}), T cells with AC (5×10^{4} each) or AC alone (5×10^{4}) in 0.2 ml-volumes per well were stimulated in triplicate with serial dilutions of SDM and SpeC in 96-well flat bottomed tissue culture plates (Beckton Dickinson and Co., Franklin Lakes, NJ, USA). After 2 days of incubation (37 ℃, 5.0% CO_2), 0.5 μCi of ^{3}H-thymidine was added to each culture, which was allowed to incubate for an additional 16 hours. Radiolabelled DNA was harvested onto

glass fiber filters with a semi-automatic harvester. The amount of radioactivity incorporated into cellular DNA was quantified by a MATRIX direct beta counter (Packard Meridien, CT, USA).

Cultivation of T cell blasts

SDM, SpeC or Con A-induced bovine T cell blasts were prepared by stimulating PBMC with 100 ng/ml of SDM and SpeC or 5 μg/ml of Con A. After 3 days harvested blasts were expanded with 100 U/ml of r-IL-2 for four days. Human T cell blasts were made in the same way but 100 ng/ml of SDM, SpeC and 50 μg/ml of anti-CD3 mAb, instead of Con A, were used as in previous reports[14].

Analysis of TCR-Vβ repertoires of human and bovine T cells reactive to SAG

The TCR Vβ usage of SAG-reactive human T cells was determined by a modified version of the RT-PCR method described by Kato et al[16] that uses twenty-six previously designed human Vβ-specific 5′ primers and a Cβ-specific 3′ primer. For the bovine RT-PCR assay fourteen bovine Vβ-specific sense primers designed by T. Hayashi coupled with an anti-sense oligomer from the downstream Cβ region were used (bovineVβBTB 4-GGACTTTCAAGCTACAACTG, bovine VβB-TB13-CAGAAAATGAATCATTATGCAAG, bovineVβBTB 18-TGTCTCTGGACACCTCTCTG , bovine Cβ GGAGATCTCTGCTTCCGAGGGT-TC*CαF-CCTGTGATGCCAAGCTGGTAGAGA, CαR-TCAACTGGACCAGAGCCGCA).

Total cellular mRNA was collected from SDM, SpeC or anti-CD3 mAb induced human T cell blasts using ISOGEN reagent (Nippon Gene KK, Tokyo, Japan). cDNA was synthesized by incubating mRNA samples with 5 units of reverse transcriptase (AMV Reverse Transcriptase XL) and random DNA hexamers (Takara Bioinc., Shiga, Japan) in a final volume of 25 μl for 1hr at 41 ℃. Each human Vβ gene was examined by taking an aliquot of cDNA and amplifying for 31 cycles of 94 ℃30 sec/55 ℃30 sec/72 ℃30 sec using one of the 26 5′ Vβ-specific sense primers and the 3′ Cβ-specific anti-sense primer with 1 unit of Taq DNA polymerase (Sigma-Aldrich) in a final volume of 20 μl. A human TCR Cα primer pair was used as a control.

In the same way, bovine cDNA was prepared from SDM-, SpeC- or Con A-induced T cell blasts. PCR reactions were performed in a 30 μl volume containing 0.2 μM of each primer and 0.7 U of Taq polymerase (Promega, Madison, WI, USA) under the following conditions: one minute each of 95 ℃ denaturation, 52 ℃ annealing and 72 ℃ extension, for 35 cycles. In each tube a co-amplification of TCR Cα cDNA served as an internal control.

The amplified products were resolved on an ethidium bromide stained 2% agarose gel and were analyzed with a densitometric image analyzer (ATTO Co., Ltd., Tokyo, Japan).

*Sequences of the remaining bovine Vβ-specific primers are available on request from T. Hayashi

Analysis of densitometry data

Densitometry values for each specific Vβ product were normalized by dividing the Vβ value with the corresponding Cα control value. Results were then represented as a percentage expression of each Vβ element calculated in relation to the sum of all the ratios, according to the following formula:

$$\%V\beta n = [V\beta_n^{norm}/\Sigma(V\beta/C\alpha)_{1\text{-}R}] \times 100$$

The value R represents the number of Vβ elements looked at: 14 for the bovine analysis and 26 for the human.

Dendrogram preparation

A dendrogram showing relationships of known human and bovine TCR-Vβ elements was constructed by means of the Search and Analysis service based on Clustal W[17] in the DDJB (http://www.ddjb.nig.ac.jp/E-mail/clustalw-e.

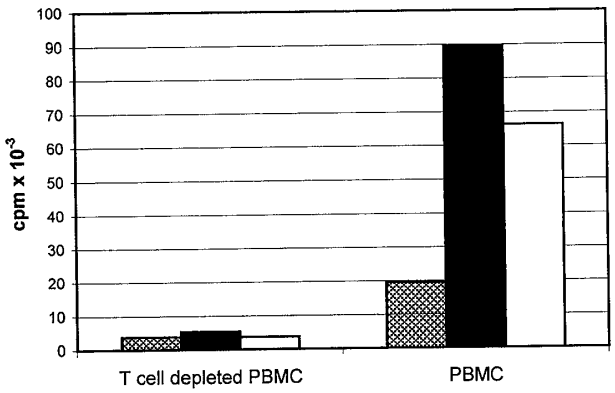


Fig. 1 T-cell-stimulating activity of SDM and SpeC
Whole and T cell-depleted bovine PBMC (1×10^5 per well) in 96 well plates were stimulated either without SAG (hatched bars) or with an optimal dose of 100 ng/ml of SDM (filled bars) or SpeC (open bars) for 2 days and then examined for mitogenic responses. Results are shown as mean [^{3}H]-thymidine uptake in counts per minute of triplicate samples.

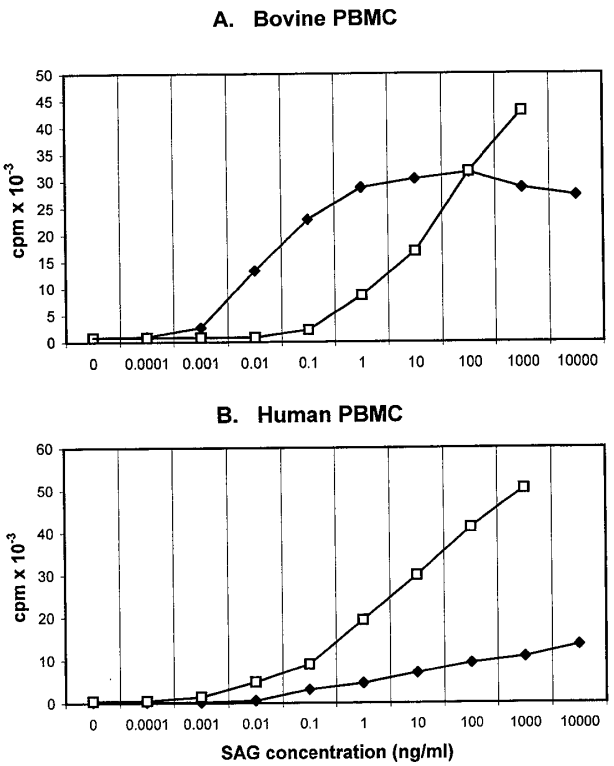


Fig. 2 Mitogenic activity of SDM and SpeC on PBMC T cells
(A) Bovine and (B) human PBMC (1×10^5 per well) in 96 well plates were stimulated with increasing doses of SDM (filled symbols) and SpeC (open symbols). Primary proliferative responses were measured by a [^{3}H]-thymidine uptake assay. Data are shown as mean counts per minute of triplicate samples and are representative of results from at least three different donors.

html). Amino acid sequences were aligned and then the dendrogram drawn, using the Treeview program[18].

Results

Identification of target cells in SDM and SpeC-reactive PBMC

It is well established that SDM and SpeC act on human PBMC by targeting CD4 and CD8 positive T cells[19]. Our preliminary investigations showed that SDM and SpeC are potent stimulators of bovine PBMC. We thus conducted experiments to identify the bovine cell type targeted by these SAGs. Whole and T cell-depleted bovine PBMC were stimulated *in vitro* with 100 ng/ml of SDM or SpeC and their respective proliferative responses measured with a ^{3}H-thymidine uptake assay. Whole PBMC proliferated vigorously while T cell-depleted cells did not, indicating that T cells are essential for this response (Fig. 1).

Comparison of SAG activity of SDM and SpeC on human and bovine T cells

To examine the effect of SDM and SpeC on bovine and human PBMC T cells, PBMCs were cultured with increasing concentrations of the SAGs and ^{3}H-thymidine uptake measured. Bovine T cell proliferation was induced by an SDM dose of 0.01 ng/ml or more (Fig. 2A). Increasing amounts of SDM resulted in increased proliferation with a maximum response at a dose of 100 ng/ml and a subsequent steady state response. SpeC induced a similar dose dependant response but at 1 ng/ml or more, a concentration one hundred times higher than SDM.

Human T cell responses were quite different from the bovine responses (Fig. 2B). SDM-induced proliferation was seen at 10 ng/ml or more, while SpeC induced proliferation was seen at doses of 0.1 ng/ml or more. The magnitude of

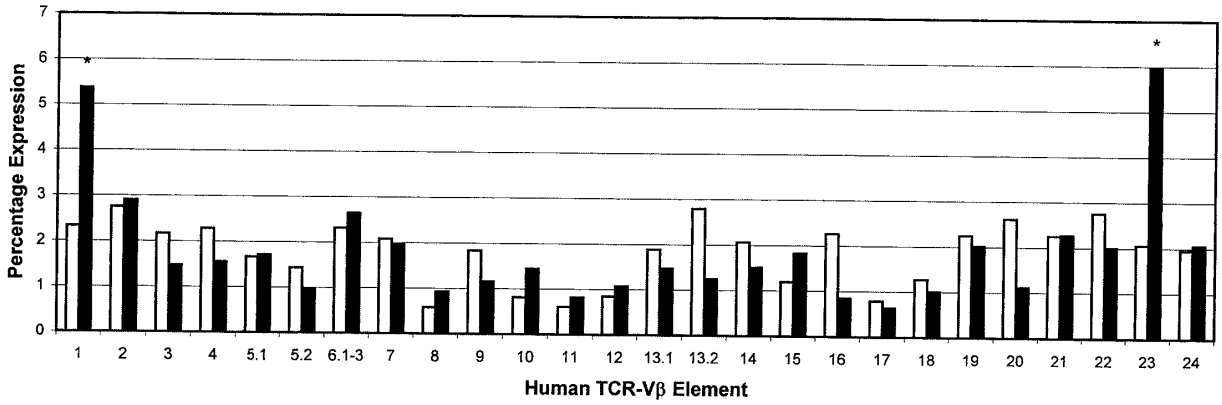


Fig. 3 TCR-Vβ repertoire of human T cells stimulated with SDM
Human T cell blasts prepared by stimulation with SDM (filled bars) or anti-CD3 antibody (open bars) were analyzed for expression of various TCR Vβ genes by RT-PCR. Data are presented as percentage expressions of individual Vβ elements. Significant increases in comparison with anti-CD3-induced expression are indicated with an asterisk.

the SpeC response was also much higher than the SDM response. These findings revealed that bovine T cells are much more sensitive to SDM than SpeC, whereas for human T cells the reverse is true.

Identification of TCR-Vβ repertoires of human T cells reactive to SDM

The human T cells targeted by SDM were previously determined to be the Vβ1 and Vβ23 positive cells by flow-cytometric analysis of SDM-induced T cell blasts labeled with anti-Vβ monoclonal antibody[14]. In this study we used an RT-PCR method incorporating as many PCR primers as possible. Because the number of anti-Vβ monoclonal antibodies available is limited, this alternative approach enabled us to examine a wider range of Vβ subtypes. SpeC-reactive human T cells were already determined to be $V\beta_2^+$ using the RT-PCR method [20].

As described in the Materials and Methods, human PBMC were stimulated with SDM or anti-CD3 mAb. T cell lymphoblasts produced were expanded in the presence of IL-2 and then subjected to RT-PCR to determine their TCR-Vβ usage. Figure 3 shows expression of individual Vβ elements as a percentage of total Vβ expression for the SDM-induced and anti-CD3 mAb-induced T cell blasts. The percentage expression of Vβ1 and Vβ23 was much higher in the SDM treated cells than the anti-CD3 mAb treated cells. For the other Vβ elements, expression levels remained about the same or else SDM T-cell blast levels were lower than the anti-CD3 levels. This confirmed our earlier findings that SDM selectively activates $V\beta1^+$ and Vβ23 + T cells and also provided assurance that SDM does not target other Vβ types that we might not have been able to detect before.

Identification of TCR-Vβ repertoires of bovine T cells reactive to SDM and SpeC

Similarly, bovine T cell blasts were prepared following stimulation of PBMC with SDM, SpeC or Con A and then subjected to RT-PCR to determine which bovine T-cells are reactive to these SAGs.

The findings illustrated in Fig. 4A are representative of the results of several repeat experiments. For the range of bovine TCR Vβ chain transcripts examined, SDM induced blasts were significantly richer in VβBTB18 mRNA than Con

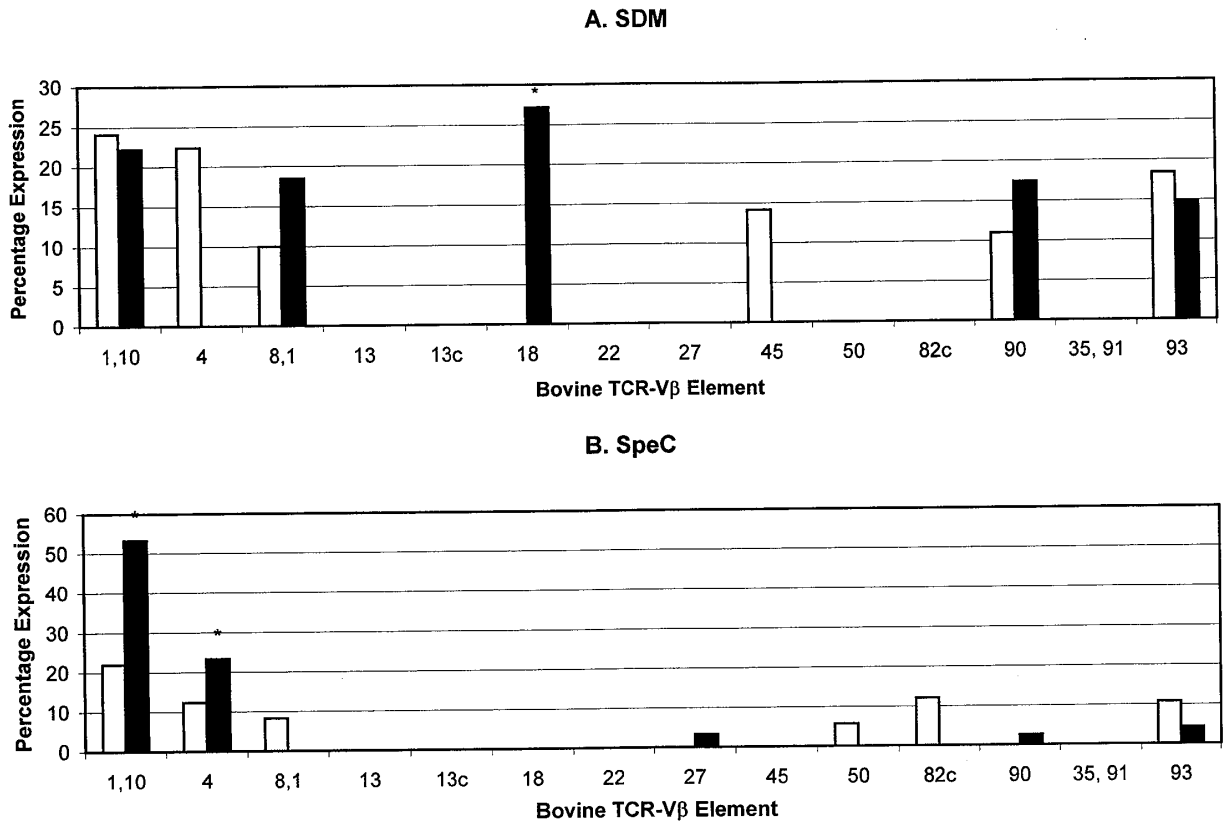


Fig. 4 TCR-Vβ repertoire of bovine T cells stimulated with SDM or SpeC
Bovine T cell blasts prepared by stimulation with SAG (filled bars) or Con A (open bars) were analyzed for expression of various TCR Vβ genes by RT-PCR. Data are presented as percentage expressions of individual Vβ elements. Significant increases in comparison with Con A-induced expression are indicated with an asterisk.

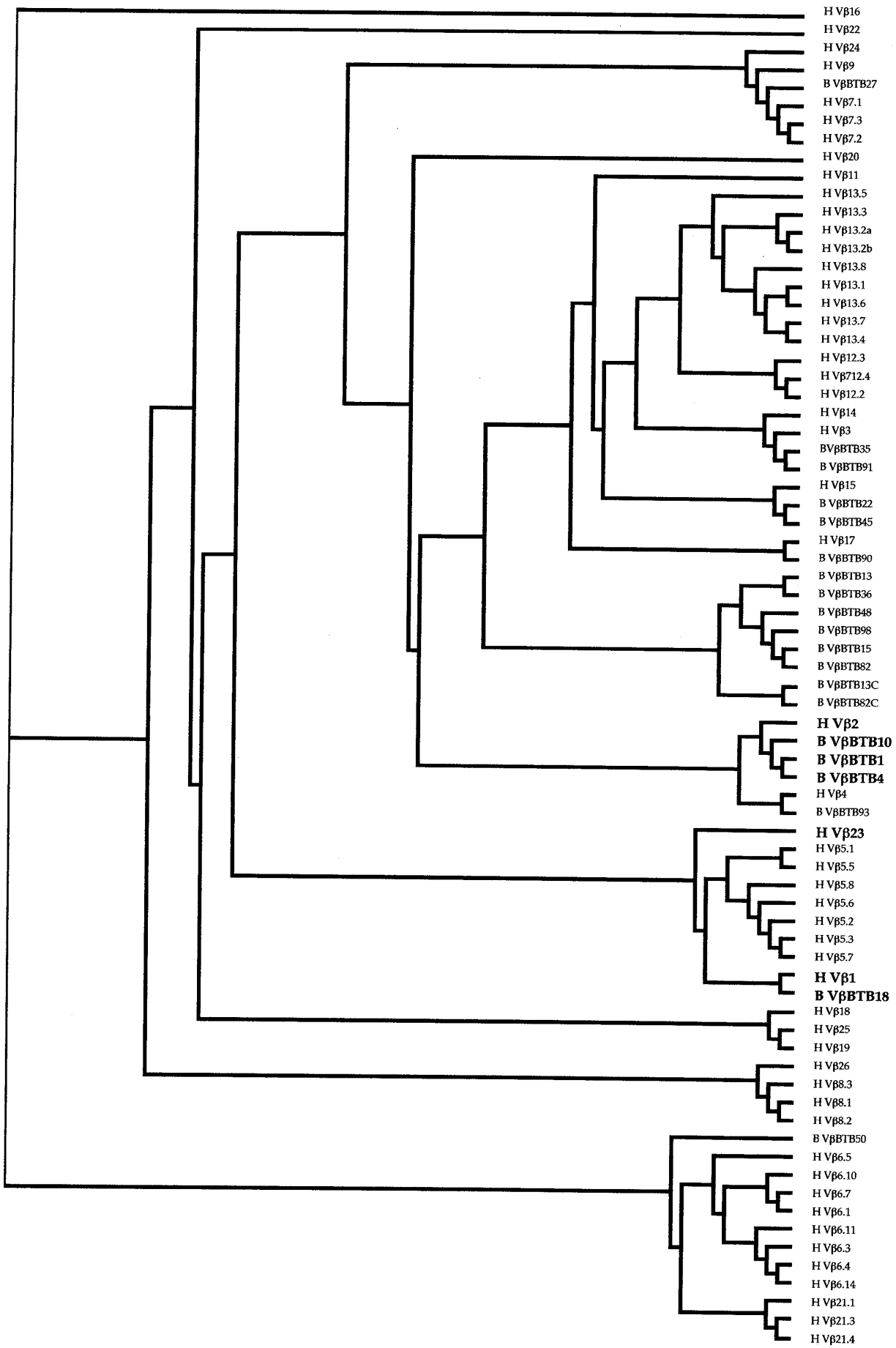
A blasts. This implied that the bovine VβBTB18 positive T cells expanded in response to the SAG. All the other detectable subsets contracted except for VβBTB8-1 and VβBTB90. The apparent expansion of these two cell types proved inconsistent upon examination of samples from other donors. Moreover, prolonging the duration of SDM mitogenic stimulation resulted in progressive expansion of VβBTB18 cells only (data not shown), evidence that none other than these respond to SDM.

Similar treatment of bovine PBMC with SpeC resulted in the expansion of bovine VβBTB1, 10 and 4 positive T cells (Fig. 4B). Note that the primer used cannot distinguish between VβBTB1 and VβBTB10.

Homology analysis of bovine and human TCR-Vβ elements

We used the Clustal W program to conduct a homology analysis with database amino acid sequences of human and bovine TCR-Vβ elements by the neighbor-joining method. The dendrogram generated (Fig. 5), shows the relationships of known bovine and human Vβ elements.

On one branch is VβBTB18, the SDM target on bovine T cells and its human homologue, Vβ1, the human SDM target. The other human target, Vβ 23, is closely related and is located on the same branch. The human Vβ5 elements are also very similar to Vβ1 and 23 and though none of them seemed to expand in our assays, Akiyama et al found that some individuals have SDM reactive


H Vβ16
H Vβ22
H Vβ24
H Vβ9
B VβBTB27
H Vβ7.1
H Vβ7.3
H Vβ7.2
H Vβ20
H Vβ11
H Vβ13.5
H Vβ13.3
H Vβ13.2a
H Vβ13.2b
H Vβ13.8
H Vβ13.1
H Vβ13.6
H Vβ13.7
H Vβ13.4
H Vβ12.3
H Vβ712.4
H Vβ12.2
H Vβ14
H Vβ3
BVβBTB35
B VβBTB91
H Vβ15
B VβBTB22
B VβBTB45
H Vβ17
B VβBTB90
B VβBTB13
B VβBTB36
B VβBTB48
B VβBTB98
B VβBTB15
B VβBTB82
B VβBTB13C
B VβBTB82C
H Vβ2
B VβBTB10
B VβBTB1
B VβBTB4
H Vβ4
B VβBTB93
H Vβ23
H Vβ5.1
H Vβ5.5
H Vβ5.8
H Vβ5.6
H Vβ5.2
H Vβ5.3
H Vβ5.7
H Vβ1
B VβBTB18
H Vβ18
H Vβ25
H Vβ19
H Vβ26
H Vβ8.3
H Vβ8.1
H Vβ8.2
B VβBTB50
H Vβ6.5
H Vβ6.10
H Vβ6.7
H Vβ6.1
H Vβ6.11
H Vβ6.3
H Vβ6.4
H Vβ6.14
H Vβ21.1
H Vβ21.3
H Vβ21.4

Vβ5 T cells [14].

Again as expected, the human SpeC target, Vβ 2, is situated in close proximity to the SpeC reactive bovine elements VβBTB1, 4 and 10, which are all clustered on one branch.

Discussion

Our experiments showed that the SAGs, SDM and SpeC, stimulate the proliferation of bovine PBMC but not CD2, CD4 and CD8 positive cell-depleted PBMC (Fig. 1). We also showed that SDM and SpeC activate T cells expressing particular TCR-Vβ elements (Fig. 4). Although it is unclear how CD2, CD4 or CD8 cell surface molecules are distributed on different bovine PBMC sub-populations, we do know that in well studied immune systems such as human and mouse systems, T cells constitute the major portion of CD2, CD4 and CD8 positive cells. We also know that SAGs target human and murine T cells. These facts considered together, indicate that SDM and SpeC act on bovine T cells.

We also demonstrated that bovine T cells are more responsive to SDM than SpeC and human cells more responsive to SpeC than SDM (Fig. 2). Several SAGs have been shown to activate the T cells of different mammals and although the sensitivity of individual animal species for specific molecules varies, the mechanism of T cell activation in the various animals is almost certainly the same[21)22)]. The intact SAG molecule binds to an MHC II molecule on an AC outside the peptide-binding groove and also to a specific TCR-Vβ chain on a T cell. Variations in effect between different SAGs and between different species are most likely due to the binding efficiency for both the MHC II and TCR sites. Although the specific reason for the difference in the reactivity of bovine and human cells was not looked for in this study, we believe that it is probably due to differences in affinity between MHC II and SAG molecules because analogous reactive T cells are present in both species (Figs. 3, 4, 5) and because several reports document that variations in the structure of these molecules can have a significant effect on their ability to bind SAG molecules[23)24)]. Differences between corresponding bovine and human MHC molecules have been described[25)]

We were interested to find that SDM, which comes from a bovine pathogen, had a stronger effect on bovine T cells than human T cells while the opposite was true of SpeC, which is derived from a mainly human pathogen. If this phenomenon is true for all species, it suggests that pathogenic organisms adapt to their natural hosts by production of SAGs that are most effective on that host. This supports the idea that SAG action is beneficial to the bacterium and gives it some long-term survival advantage. Probably, SAGs hinder host efforts to eliminate the producing organism by disturbing the host immune response[26)].

In humans, the uncoordinated release of pro-inflammatory cytokines such as IL-2, IFN-γ and in particular TNF-α are believed to be responsible for many of the clinical features of SAG diseases, particularly the systemic shock syndromes[7)~10)]. We found that these same cytokines could be pro-

Fig. 5 Relationship between bovine and human TCR-Vβ elements of SDM and SpeC responsive T cells

Amino acid sequences of known bovine and human Vβ elements stored in the DNA Data Bank of Japan (DDJB) were aligned using the Clustal W program and a dendrogram constructed by the neighbor joining method in this program. The tree, drawn by the TreeView program, shows responsive Vβ elements in bold print.

duced by bovine cells through the action of SDM (data not shown). Though similar manifestations of disease during bovine *S. dysgalactiae* infections have not yet been documented, it is quite possible that SDM could cause a toxic shock-like syndrome in cows. *In vivo* studies on the effects of SDM on the cow are necessary to get a clearer picture, particularly since preliminary *in vivo* studies on another mammal, the rabbit, have demonstrated the toxic effect of this molecule (unpublished data).

We do know that *S. dysgalactiae* infections in cows are associated with bovine mastitis. SpeC producing *S. pyogenes* is also a frequently isolated pathogen in this condition [27)28)]. The pathological processes occurring during bovine mastitis are not yet well understood and it is uncertain whether SAGs are involved [29)]. It has been suggested however, that SAGs have a role even in unremarkable local infections with SAG producing organisms like *S. pyogenes* pharyngitis in humans[30)]. It is thought that the spectrum of SAG disease probably results from differences in both the rate and site of superantigen production, and the immune response of the host. By the same token, SDM may have a local effect in bovine mastitis.

Acknowledgments

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References

1) **Li H, Llera A, Malchiodi EL et al**: The structural basis of T cell activation by superantigens. Annu Rev Immunol **17**: 435–442, 1999
2) **UchiyamaT, Yan XJ, Imanishi K et al**: Bacterial superantigens-mechanism of T cell activation by the superantigens and their role in the pathogenesis of infectious diseases. Microbiol Immunol **38**: 245–256, 1994
3) **Dinges MM, Orwin PM, Schlievert PM**: Exotoxins of *Staphylococcus aureus*. Clin Microbiol Rev **13**: 16–34, 2000
4) **Fraser J, Arcus V, Kong P**: Superantigens—powerful modifiers of the immune system. Mol Med Today **6**: 125–132, 2000
5) **Cunningham MW**: Pathogenesis of group A streptococcal infections. Clin Microbiol Rev **13**: 470–511, 2000
6) **Uchiyama T, Miyoshi-Akiyama T, Kato H et al**: Superantigenic properties of a novel mitogenic substance produced by *Yersinia pseudotuberculosis* isolated from patients manifesting acute and systemic symptoms. J Immunol **151**: 4407–4413, 1993
7) **Miethke T, Wahl C, Regele D et al**: Superantigen mediated shock: a cytokine release syndrome. Immunobiology **189**: 270–284, 1993
8) **McCormick JK, Yarwood JM, Schlievert PM**: Toxic shock syndrome and bacterial superantigens: an update. Annu Rev Microbiol **55**: 77–104, 2001
9) **Norrby-Teglund A, Chatellier S, Low DE et al**: Host variation in cytokine responses to superantigens determine the severity of invasive group A streptococcal infection. Eur J Immunol **30**: 3247–3255, 2000
10) **Norrby-Teglund A, Thulin P, Gan BS et al**: Evidence for superantigen involvement in severe group A streptococcal tissue infections. J Infect Dis **184**: 853–860, 2001
11) **Stevens DL, Tanner MH, Winship J et al**: Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. N Engl J Med **321**: 1–7, 1989
12) **Takahashi N, Nishida H, Kato H et al**: Exanthematous disease induced by toxic shock syndrome toxin 1 in the early neonatal period. Lancet **351**: 1614–1619, 1998
13) **Takahashi N, Kato H, Imanishi K et al**: Immunopathophysiological aspects of an emerging neonatal infectious disease induced by a bacterial superantigen. J Clin Invest **106**: 1409–1415, 2000
14) **Miyoshi-Akiyama T, Zhao J, Kato H et al**: *Streptococcus dysgalactiae*-derived mitogen (SDM), a novel bacterial superantigen : characterization of its biological activity and predicted tertiary structure. Mol Microbiol **47**: 1589–1599, 2003
15) **Uchiyama T, Kamagata Y, Yan XJ et al**: Study of the biological activities of toxic shock syndrome toxin-1: II. Induction of the proliferative response and the interleukin 2 production by T

cells from human peripheral blood mononuclear cells stimulated with the toxin. Clin Exp Immunol **68**: 638–647, 1987

16) **Kato H, Fujimaki W, Narimatsu H et al**: Study of TCR Vβ usage in superantigen-reactive human T cells by the RT-PCR method. J Tokyo Wom Med Univ **64**: 985–993, 1994

17) **Thompson JD, Higgins DG, Gibson TJ**: Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res **22**: 4673–5480, 1994

18) **Page RD**: An application to display phylogenetic trees on personal computers. Comput Appl Biosci **12**: 357–438, 1996

19) **Yan XJ, Li X, Imanishi K et al**: Study of activation of murine T cells with bacterial superantigens. J Immunol **150**: 3873–3881, 1993

20) **Li P, Tiedemann RE, Moffat SL et al**: The superantigen streptococcal pyrogenic exotoxin C (SpeC) exhibits a novel mode of action. J Exp Med **186**: 375–383, 1997

21) **Pettersson H, Forsberg G**: Staphylococcal enterotoxin H contrasts closely related enterotoxins in species reactivity. Immunology **106**: 71–79, 2002

22) **Bavari S, Hunt RE, Ulrich RG**: Divergence of human and nonhuman primate lymphocyte responses to bacterial superantigens. Clin Immunol Immunopathol **76**: 248–254, 1995

23) **Imanishi K, Igarashi H, Uchiyama T**: Relative abilities of distinct isotypes of human major histocompatibility complex class II molecules to bind streptococcal pyrogenic exotoxin types A and B. Iufect Immun **60**: 5025–5029, 1992

24) **Welcher BC, Carra JH, DaSilva L et al**: Lethal shock induced by streptococcal pyrogenic toxin A in mice transgenic for human leukocyte antigen DQ8 and human CD4 receptors: implications for development of vaccines and therapeutics. J Infect Dis **186**: 501–510, 2002

25) **Morooka A, Asahina M, Kohda C**: Nucleotide sequence and the molecular evolution of a new A2 gene in the DQ subregion of the bovine major histocompatibility complex. Biochem Biophys Res Commun **212**: 110–117, 1995

26) **Llewelyn M, Cohen J**: Superantigens: microbial agents that corrupt immunity. Lancet Inf Dis **2**: 156–162, 2002

27) **Watts JL**: Etiological agents of bovine mastitis. Vet Microbiol **16**: 41–66, 1988

28) **Schuberth HJ, Krueger C, Zerbe H et al**: Characterization of leukocytotoxic and superantigen-like factors produced by *Staphylococcus aureus* isolates from milk of cows with mastitis. Vet Microbiol **82**: 187–199, 2001

29) **Calvinho LF, Almeida RA, Oliver SP**: Potential virulence factors of *Streptococcus dysgalactiae* associated with bovine mastitis. Vet Microbiol **61**: 93–110, 1998

30) **Bernal A, Proft T, Fraser JD et al**: Superantigens in human disease. J Clin Immunol **19**: 149–157, 1999

Streptococcus dysgalactiae 由来マイトジェン（SDM）のウシT細胞に対するスーパー抗原活性の検討

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Streptococcus dysgalactiae 由来マイトジェン（SDM）は近年，当教室でウシ由来菌株 *S. dysgalactiae* より精製され，ヒトT細胞に対してスーパー抗原活性を示す物質である．SDMがウシ由来菌株より得られた物質であることから，ヒトよりウシT細胞に強く働くスーパー抗原であることを予測し，実験を行った．SDMはウシ末梢血中のT細胞の細胞増殖を誘導した．ヒトではT細胞レセプター(TCR)にVβ1あるいはVβ23を持つT細胞を活性化する．SDMはこれらのVβに類似した構造を持つウシのVβBTB18陽性T細胞を活性化した．以上により，SDMはウシT細胞に対してスーパー抗原活性を示すことが明らかになった．SDMのスーパー抗原活性をヒトに病原性を示す *S. pyogenes* が産生するスーパー抗原SpeCと比較した．SDMにはウシ末梢血が，SpeCにはヒト末梢血がより強い細胞増殖反応を示した．SDMはウシの *S. dysgalactiae* 感染症において病原因子としてより大きな役割を持つことが示唆された．