The JOURNAL
of
APPLIED RESEARCH
In Clinical and Experimental Therapeutics

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Role of Streptococcus sanguis and Traumatic Factors in Behçet's Disease

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KEY WORDS: Behçet's disease, Streptococcus sanguis, experimental model, germ-free mouse, ocular inflammation.

ABSTRACT

Background: The pathogenicity of Behçet's disease (BD) has been associated with the offending pathogen Streptococcus sanguis. However, it is unclear that the bacterium is a true pathogen.

Materials and Methods: Germ-free mice were inoculated with a clinical isolate (strain BD113–20) of S. sanguis. Mice received heat or mechanical stress on their oral tissue before the bacterial infection. Colonization, immune responses against the cell wall and synthetic peptides, and the cytokine profile were examined. Uveitogenicity of the cell wall, lipoteichoic acid (LTA), muramyl dipeptide (MDP), human hsp336–351, S. sanguis-associated peptides, and retina-associated peptides were examined.

Results: S. sanguis colonized the oral cavity at 105–8/mL saliva. The level of colonization in mice given heat or mechanical stress was significantly higher than the other groups. These mice showed typical oral ulcers after the bacterial challenge and mild iridocyclitis. Skin lesions were spread whereas genital ulcers were rare in these groups. Significant antibody production to the selected peptides was observed in the experimental mice compared with control animals. Inflammatory cytokines such as IL-2, IL-6, IFN-g, and TNF-a were detected in oral tissue of the mice infected with S. sanguis. Evidence suggests that the association with the cell wall or with LTA can directly affect the degree of inflammation.

Conclusions:

S. sanguis strain BD113–20 is pathogenic for experimental mice and can be a causative agent for BD. Molecular mimic peptides can be implicated in the pathogenesis of BD. The cell wall of the bacteria shows direct ocular inflammogenicity.

Introduction

Behçet's disease (BD) is a multisystemic disorder presenting with recurrent oral and genital ulceration as well as uveitis, often leading to blindness.1,2 The etiology and pathogenesis of this syndrome remains obscure. We found that the proportion of S. sanguis in the oral flora of patients with BD has significantly increased compared with control subjects.3,4 Patients show hypersensitivity in skin tests with the streptococcal antigens, and symptoms typical of BD are sometimes provoked by an injection of the antigen.5 Recently, we showed antibody crossreactivity from sera of patients with BD with synthetic peptides that have homologies with proteins from S. sanguis.6

The concept of overexpression of hsp either on the cell surface proper or as peptides presented by MHC products has been central to the

hypothesis that hsp-specific antibodies and T cells play a role in the pathogenesis of human autoimmune disease. T cell response to hsp60 and increased levels of hsp-60-specific antibodies in serum have been found in patients with BD.8,9 These immune responses to hsp have also been found in a number of human autoimmune diseases.7,9 Thus, it is difficult to explain the pathogenesis of BD by hsp alone, even if human hsp is homologous with hsp from S. sanguis.

Recently, we succeeded in the isolation and sequence determination of the bes-1 encoding a streptococcal antigen that correlated with BD.10 The residues in a portion of the amino acid sequence show 60% similarity to the human intraocular peptide Brn-3b. Brn-3, a subfamily of POU (pit-Oct Unc) domain factors, contain three members, Brn3a, Brn3b, and Brn3c.11 POU domain proteins are a class of transcriptional regulators that appear to have important roles in tissue-specific gene regulation. Pit1 plays a critical role in the development of the pituitarity and regulation of prolactin and growth hormone synthesis; Oct1 is an ubiquitous transcription factor and Oct2 regulates immunoglobulin synthesis in B lymphocytes. Brn-3b is first expressed in migrating, postmitotic ganglion cell precursors in the ventricular zone of developing mouse retinas.12

Fox et al.13 noted that peptidoglycan provoked chronic inflammation and retinal necrosis similar to that observed in eyes injected with lipopolysaccharide. However, because of the crude nature of the cell wall extracts, the specific basis for cell wall-induced inflammation was not determined. It has been reported that Gram-positive cell walls stimulate synthesis of tumor necrosis factor alpha and interleukin-6 by human monocytes.14 With regard to the intraocular inflammogenicity of the cell wall, neither the metabolically inactive pathogens nor purified sacculi caused significant reductions in retinal responsiveness, but they evoked significant inflammation in both the posterior and anterior segments of the eye.15

The aims of this study are to produce an experimental model with mono-infection of S. sanguis by using germ-free mice. Under the gnotobiotic condition, the effect of other microbiota can be eliminated. We also examined whether the cell wall and its constituents are capable of inciting significant intraocular inflammation because the determination of the virulence factor is important.

MATERIALS AND METHODS

Bacterial Strain Used

S. sanguis strain BD113-20 was used for the experiments. The strain was isolated from the oral cavity of a patient with BD3. A similar serotype (so-called KTH-1) was found in more than half of patients with BD, but not in healthy control subjects.4 Clinical isolates belonging to serotype KTH-1 has been identified as S. oralis by their biochemical and enzymatic properties.16 However, our isolates were different from those strains in the analysis of DNA homology and cell wall sugar constituents.17 Bacteria were cultured in BHI medium at 37–C for 24 hours.

Preparation of the Cell Wall and Other Components

The S. sanguis cell wall was prepared by the method previously described.18 LTA from S. sanguis and MDP were purchased from Sigma Chemical Co. (St. Louis, MO). They were suspended in sterile Hanks' balanced salt solution (Sigma Chemical Co.) at a concentration of 1 mg/mL.

Mice

Germ-free IQI/Jic mice, bred from ICR mice, were obtained from Japan Clea Co. Ltd. (Tokyo, Japan). Ninety-five female and male mice were used at 4 to 5 weeks of age. Each infection group consisted of 10 mice whereas the negative control groups consisted of 5. Mice were maintained at biohazard level 3. Food and drinking water were autoclaved before use. Before and after the experiments, feces and bedding were cultured on BHI agar with 7% horse blood under both anaerobic and aerobic conditions. No bacteria were contaminating the culture at any time. Specific pathogen-free ICR mice (males, 5 wk of age) were used for the experiments to confirm uveitogenicity of various bacterial components and synthetic peptides. These animals were fed with standard laboratory chow and maintained in the standard light-dark cycle. Animals were cared for in accordance with the ARVO Statement for Use of Animals in Ophthalmic and Vision Research. The ethical committee of the Health Sciences University of Hokkaido allowed the design of our experiments.

Heat-Shock Treatment

Heat shock (HS) to induce severe inflammation was performed on the left side of the tongue dorsum and buccal surface of germ-free mice before S. sanguis infection. A spatula was preheated at 250˚C in a heat-box (Inotech STDRI 250, Inotech Co. Ltd., Tokyo, Japan), cooled to 180–200˚C (the temperature was estimated as radial energy by spot thermometer HT-7, Minorta Co. Ltd.), and then attached to the surface of the oral tissues for 10 seconds under anesthesia by using Nembutal (Dainippon Pharmaceutical Co. Ltd., Osaka, Japan). The treated area was 5 mm x 5 mm. The group undergoing heat-shock treatment was designated as the HS group.

Mechanical Damage of the Mucosal Surface (Scraping: SCR)

This method was used as an induction of mild trauma on the oral mucosal surface. The oral surface (the left side of the tongue dorsum and buccal surface, 5 mm x 5 mm) was scraped by a dental end excavator under anesthesia. The group with mechanical damage of the mucosal surface was designated as the SCR group.

Mouse Colonization Experiments

In the HS or SCR group, S. sanguis strain BD113–20 was inoculated into the oral cavity of germ-free IQI mice. These groups were designated as the HS/bac or SCR/bac group, respectively. Bacterial suspension (103, 105, 107/mouse) was deposited intraorally through a soft polyethylene catheter. Immediately after inoculation the catheter was removed and no further manipulations were performed. Control mice received PBS. After bacterial inoculation, salivary samples were collected from each mouse (1, 3, 7, and 14 days after infection) and suspended at a concentration of 10% in BHI medium and placed on MS and BHI agar plates as previously described.4 In this investigation, colonization was assessed by determining the level at which a strain persisted in the saliva. The bacteria detection limit for assessment of colonization was 102 CFU/mL. Culture of oral tissues was carried out directly on BHI agar. Feces were also obtained for monitoring the quality of the mice and colonization of S. sanguis. Bacterial counts were performed as described previously in this article.

Delayed-Type Hypersensitivity (DTH) Reaction Against the Cell Wall

Fourteen days after infection, the mice were challenged in both hind foot pads with 20 µL of a solution containing 0.5 mg/mL of the

cell wall (final 10 μg/mouse) or PBS. One foot pad (right) received the antigen and the other (left) PBS. The thickness of the foot pads (right-left) was measured before and after challenge. Control mice received only PBS to both foot pads.

Immune Responses Against
Synthetic Peptides

A peptide derived the sequence of the human hsp 336–351(QPHDLGKVGEVIVTKDD) that has been reported to stimulate T lymphocytes of patients in Japan19 was produced by the American Peptide Company (California, USA). Four other peptides, including Brn-3b of retinal ganglion cells and Bes-1 of 95-kD protein in S. sanguis,10 were also used. Common sequences were observed between Brn-3b (11–25; AFSMPHGGSLHVEPK) and Bes-1 (229–243; QPHDLGKVGEVIVTKDD) and between Brn-3b (177–189; HHHHHHHQPHQAL) and Bes-1 (373–385; HGDHHHFIPYDKL), respectively. Peptide was coated on 96 well plates using a peptide coating kit (Takara, Tokyo, Japan). Antibody titer was estimated by ELISA.

Cytokine Assay

Cytokine assay (IL-2, IL-6, TNF-a, IFN-g) was done by ELISA. Briefly, samples from the oral soft tissues (approximately 0.1-0.2g) were aseptically removed from the mice. They were suspended at a concentration of 0.1 g/mL in RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY) containing 1% (W/V) 3-([cholamidopropyl]dimethylammonio)-1-propanesulfonate (CHPA; Wako Pure Chemical Co., Kyoto, Japan) and homogenized by a tissue homogenizer (Micro Multi Mixer, Ic-Ieda Co., Tokyo, Japan). Homogenates were left on ice for 1 hour and clarified by centrifuging at 2000 x g for 20 minutes. The organ extracts were stored at -80°C until cytokine assays were undertaken. Cytokines were quantified with ELISA kits (Genzyme, Cambridge, MA). The dose was determined by a standard curve and expressed as pg/0.1g of tissue.

Intraocular Injection of Bacterial Components and Synthetic Peptides

Intraocular injection was performed with modification of the method of Callegan et al.15 We changed the needle and syringe from normal to the gas-chromatography type. Cell wall, LTA, MDP, and synthetic peptides were prepared for animal administration (0.2 μL: 0.2 μg/eye of mouse) and injected into an experimental eye of ICR mice, respectively. Care was taken to avoid traumatizing the lens during injection. In the contralateral eyes, the same volume of sterile Hanks' balanced salt solution was injected in the same manner as for the control. Five animals (5 eyes) were included in each group of the experimental or control group.

Histopathologic Examinations

Fourteen days after infection, tissue specimens were collected for histologic examination. Specimens were fixed in 10% buffered neutral formalin and processed by standard procedures. Sections of paraffin-embedded tissue were stained with hematoxylin and eosin.

Statistics

The results were expressed as mean ± standard deviation (SD). Differences between experimental and control groups were determined using the Mann-Whitney test and $P < 0.05$ was taken as the level of significance. Spearman's rank correlation was used for antibody titers against synthetic peptides.

RESULTS

Colonization of S. sanguis

Mice were divided into 10 groups as shown in Table 1. Mice were inoculated with S. sanguis after heat-shock or mechanical damage on the oral mucosal surface (designated as HS/bac and SCR/bac, respectively). The group of mice not undergoing any treatment before the bacterial infection was designated as the Bac group. The HS, SCR, and control groups corresponded to treatment (HS or SCR) alone and the negative control group. Inoculum size was shown as log10 number. In the HS/bac(7) group (inoculum size: 107/mouse), the bacteria colonized the oral cavity at 107.76–7.86/mL saliva within 7days after infection (Table 1). In the HS/bac(5) group (inoculum size: 105/mouse) and HS/bac(3) group (inoculum size: 103/mouse), colonization was persistent throughout the observation. There were significant differences in colonization between the HS/bac and bac groups until 7days after bacterial inoculation ($P < 0.05$).

In the SCR/bac(7) group (inoculum size: 107/mouse), the bacteria colonized the oral cavity at 105.74-7.22/mL saliva. There were significant differences in colonization between the SCR/bac and bac groups until 7days after bacterial inoculation ($P < 0.05$). The number of bacteria gradually decreased to that observed in control animals. The bacterial number in the SCR/bac group was lower than that in the HS/bac group for the observation period.

In the HS, SCR, and control groups, no bacteria (including S. sanguis) was detected in the saliva. S. sanguis was detected in the feces. However, the number of bacteria was similar after inoculation, ie, 7.5 ± 0.16 (log10, mean ± SD) and 7.4 ± 0.14 (log 10, mean ± SD) in the HS/bac(7) group and bac(7) groups, respectively. The other HS/bac(5), HS/bac(3), bac(5), or bac(3) groups had a similar number of bacteria in their feces.

DTH Response Against S. sanguis

The DTH reaction was used to study T cell response to S. sanguis cell wall antigen in vivo. Ten days after infection, DTH reaction was induced by injecting S. sanguis antigens into the hind foot pad (Fig. 1). Foot pad swelling was monitored for 24 hours. DTH reaction in the HS/bac groups was significantly higher than that of the others, including control animals ($P < 0.05$).

Antibody Response Against
Synthetic Peptide

Synthetic Peptide

IgG antibody titer of the tested sera against the synthetic peptides was assayed by ELISA (Fig. 2). As shown in Fig. 2A, B, and C, S. sanguis infection induced an antibody response against the peptides hsp 336–351, Brn-3b 11–25, and Bes-1 229–243, respectively. There were significant differences between the HS/bac or bac group and control animals ($P < 0.05$). Antibody titer was dependent on the inoculum size of S. sanguis, especially in the response against hsp 336–351 (Fig. 2A). The HS/bac group showed a higher antibody response against Brn-3b 11–25 and Bes-1 229–243, but there was no significant difference among the HS/bac, bac, and SCR/bac groups. In these groups, antibody responses against Brn-3b 177–189 and Bes-1 373–385 (different epitopes from Brn-3b and Bes-1) were also seen. No antibody response was observed in the HS, SCR, and control groups. Correlation among the antibody responses against hsp, Brn-3b, and Bes-1 was recognized ($P < 0.01$). Correlation coefficient in the antibody response ranged from 0.581 to 0.861, as shown in Table 2.

Detection of Cytokine

Inflammatory cytokines such as IL-2, IL-6, IFN-g, and TNF-a were detected in oral tissue of the mice infected with S. sanguis (Table 3). The HS/bac group showed strong local cytokine responses. However, the SCR/bac and bac groups showed only minimal cytokine responses, with some levels being similar to those in control animals.

Histopathologic Examination in Gnotobiotic Mice With S. sanguis

Oral tissues of the HS/bac group showed continuous severe inflammation with cellular infiltration. Microhemorrhages and edematous changes of the capillary endothelia were observed in the group. A mouse, which died at 5 days after 107 bacterial inoculation, showed severe intestinal and genital ulcerations with associated bleeding. Skin lesions were observed in the HS/bac group. An erythema arose at the site of inoculation of the bacteria and seemed to be related to severity of oral ulceration. The skin lesion continued to enlarge until 7 days after bacterial inoculation, after which it gradually disappeared. The peripheral ring-like erythema lesions were approximately 1-2 cm in width, with a range of 0.5–4 cm, but differed from erythema nodosum or erythema multiforme seen in the patients with BD. These lesions were characterized in the HS/bac group.

Oral tissue in the SCR/bac group showed mild inflammation. Oral epithelial degeneration and some polymorphonuclear leukocytes were seen in the lesion. The skin lesion was limited around the trauma area. No systemic diseases were observed in these animals.

The Bac group showed only limited oral epithelial degeneration with infiltration of a small number of polymorphonuclear leukocytes. The mice had no systemic diseases. There were no obvious clinical signs (weight loss at the site of necropsy, diarrhea, and others) as a result of the stress alone.

Mice inoculated with S. sanguis showed mild anterior segment inflammation such as ocular lesions (Fig. 3). This lesion was not only in HS/bac group, but also the bac group and SCR/bac group. However, we could not induce posterior segment inflammation. A microscopic examination revealed mild polymorphonuclear leukocyte infiltration in the affected sites. The lesion was not observed in control animals.

Contribution of Bacterial Components and Peptides to Intraocular Inflammation

To assess the relative contributions of bacterial components and peptides to ocular inflammogenicity, cell wall, LTA, MDP, and synthetic peptides were injected into the eye. CW, LTA, and MDP showed ocular inflammogenicity at 24 hours (Fig 4). The inflammatory cells and fibrin were observed in the anterior chamber. Hemorrhages were also seen at the affected sites. At 6 hours after injection of these bacterial products, the number of inflammatory cells in the eye was slight. Synthetic peptide Brn-3b induced cellular inflammation at 24 hours (Fig. 5) but not 6 hours. After injection of hsp336–351 or Bes-1, histologic features were similar to their controls (Fig. 5).

DISCUSSION

The possibility of a role for S. sanguis in BD has been raised by several observations.3,5,9,19 In this study, it is clear that S. sanguis infection after oral heat trauma in germ-free mice can induce oral and ocular diseases similar to BD. Thus, it seems that the severity of oral tissue damage is important to trigger the disease. After colonization, another step such as ulcer formation could be required before the potentially harmful systemic events can occur. We think at least the following three conditions must be in place: 1) significant antigenic mimicry between the microbe and host, 2) an abnormal cellular and humoral response on the part of the host to the microbial antigens cross-reactive with tissue antigens, and 3) genetic factors that favor an abnormal host response to cross-reactive antigens. The trauma appears to be the turning point as to whether the S. sanguis infection is limited to a local site or expanded to a systemic level.

In this study, mice colonized by the organisms showed a delayed hypersensitivity foot pad reaction against the bacterial antigen. Oral trauma such as heat damage can enhance the entrance of the bacteria; in the mouse model, the number of colony counts was significantly elevated. DTH reaction against the cell wall, high inflammatory cytokine levels, and severe damage of oral tissue were demonstrated only in the HS/bac group. High levels of colonization would lead to severe inflammatory responses.

Bacterial adherence to mammalian cells is thought to be the first step in the process leading to infection. We described that the adhesion of S. sanguis to the buccal epithelial cells from patients with BD was different from that in healthy control subjects.20 The epithelial cells exposed to S. sanguis exhibited varying and identifiable degrees of adhesiveness for the organisms in patients with BD. S. sanguis easily adhered to the degenerative cells. In our mouse model, HS treatment enhanced the bacterial colonization. This fact could be reflected in patients with BD.

The animals showed oral ulceration with mild secondary anterior segment inflammation but no other signs of BD. Only one mouse, which died 5 days after infection in the HS/bac(7) group, showed severe intestinal and genital ulcerations. Skin lesions in the mice were different from those in patients with BD. The human major histocompatibility complex encodes highly polymorphic HLA responsible for antigen presentation to T cells, and BD is known to be strongly associated with a particular HLA-B allele, HLA-B51.21 Mice do not possess these disease-susceptible genes. In the progressive stage such as posterior segment inflammation, these disease-susceptible genes could be needed.

It has been reported that hsp, specifically amino acid sequence 336–351, is an important antigen.8,9,22,23 Our mouse model showed infection of S. sanguis induced an immune response against the synthetic peptide. This response depended on bacterial inoculum size. Involvement of hsp in autoimmune responses depends on two criteria; first, hsp needs to be expressed by cells of the target organ in a different form from that at other tissue sites to allow organ-specific recognition by T cells and antibodies, and second, control of natural regulatory mechanisms for organ-specific inflammation must be disturbed.7 Pathogenic role of hsp peptides has not been accepted.2 In this experiment, direct ocular inflammogenicity was not observed in mice injected with hsp336–351. However, the specificity of the hsp peptides for BD can be applied as a diagnostic test.8 In a recent study, we extracted cellular DNA of S. sanguis from a patient with BD and cloned the fragments.10 At least two peptides were recognized as antigenic common determinants in both human cases6,10 and this mouse model. Antibody titer against Brn-3b and Bes-1 correlated with the titer against the hsp peptide. Our results showed the molecular mimic peptides can induce autoimmune-like responses.

During retinogenesis, retinal progenitors undergo a series of changes in competence to give rise to the seven classes of retinal cells present in the adult retina.24 The ganglion cells are the sole output neurons in the retina that relay light information detected by the photoreceptors to the brain. It has been suggested that there are critical roles for the Brn-3 POU domain transcription in the promotion of ganglion cell differentiation and in maintenance of differentiated ganglion cells.11

Brn3b can also mediate some of the effects that FGF2, TGFß1, and retinoic acid have on neurons.25 Antibody against Brn-3b could mean that antibody-mediated immunopathogenesis is present in BD or that there could be only crossreactive results after S. sanguis infection. We do not know Brn-3b expression or its function in BD. Brn-3b showed ocular inflammogenicity but not Bes-1. There could be some association between Brn-3b abnormalities and progression of neuro-BD. Further studies are needed.

The flora and metabolites have been found to contribute to health and diseases. Before this experiment, we examined the potential ability of colonization various anaerobes in the members of normal human flora. They colonized in the germ-free mouse but did not induce mucocutaneous ocular lesions. Mono-infected S. sanguis in this study (without any flora) induced lesions similar to BD through colonization on oral mucosal surfaces. This model can help us understand some of the unusual and as yet unexplained features of BD. One of the key aspects of the model is the prominent role played by environmental factors in the early stages. The tissue tropism of the disease could result from restrict exposure to environmental trigger through some bacterial agents. Cumulative exposure resulting in toxic levels being achieved only after many years could explain the age of onset of BD.

CONCLUSIONs

The tentative findings provide that a part of S. sanguis is pathogenic and can be a causative agent for BD. Molecular mimic peptides can be implicated in the pathogenesis of BD. Cell wall of the bacteria shows direct ocular inflammogenicity.

Acknowledgments

The authors thank Dr. Lynn Hyghes, Department of Bacteriology, Okayama University Graduate School of Medicine and Dentistry, for the critical reading of the manuscript. Supported by a research grant from the Behçet's Disease Research Committee of Japan of the Ministry of Health and Welfare of Japan.

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Table 1. Colonization of S. sanguis to Germ-Free Mice

		Number (Log10 CFU/mL)* of S. sanguis in the Saliva After Infection			
Group	Inoculum Size	1 Day	3 Days	7 Days	14 Days
HS/bac	10^7	7.76 ± 0.30†	7.86 ± 0.56†	7.84 ± 0.12†	6.12 ± 0.77†
SCR/bac	10^7	7.22 ± 0.20†	7.02 ± 0.21†	6.67 ± 0.15†	5.74 ± 0.37†
Bac	10^7	5.95 ± 0.45	6.51 ± 0.12	6.36 ± 0.67	5.06 ± 0.37
HS/bac	10^5	7.40 ± 0.43†	8.01 ± 0.02†	7.82 ± 0.41†	6.01 ± 0.64†
Bac	10^5	5.25 ± 0.48	6.22 ± 0.20	5.47 ± 0.47	5.96 ± 0.22
HS/bac	10^3	6.86 ± 0.46†	8.03 ± 0.12†	7.48 ± 0.07†	5.90 ± 0.46†
Bac	10^3	5.48 ± 0.72	5.81 ± 0.08	6.28 ± 0.25	5.59 ± 0.32
HS	0	0	0	0	0
SCR	0	0	0	0	0
Control	0	0	0	0	0

*Data were expressed as mean ± standard deviation.

†Significantly higher than the level of each bac group.

Table 2. Coefficient Among the Antibody Responses Against the Synthetic Peptides

	Hsp	Brn-3b	Bes-1	Bes-1	Brn-3b
Coefficient	336–351	11–25	229–243	373–385	177–189
Hsp 336–351	1.000				
Brn–3b 11–25	0.380*	1.000			
Bes–1 229–243	0.479*	0.496*	1.000		

Bes–1 373–385	0.165	0.325*	0.257	1.000	
Brn–3b 177–189	0.044	0.567*	0.301	0.264	1.000

*P < 0.05.

Table 3. Detection of Cytokines in Oral Soft Tissue of Germ-Free Mice Infected With S. sanguis BD113–20

		Cytokine Level* (pg/0.1 g)			
Group	Inoculum Size	IL-2	IL-6	IFN-g	TNF-a
HS/bac	10^7	43.6 ± 19.1†	60.5 ± 40.5†	62.0 ± 30.4†	56.3 ± 33.0†
SCR/bac	10^7	<15.0	17.0 ± 6.7	22.7 ± 8.0	<10.0
Bac	10^7	<15.0	13.5 ± 8.8	17.2 ± 6.8	16.3 ± 10.8
HS/bac	10^5	45.2 ± 21.8†	74.2 ± 43.4†	51.0 ± 30.3†	65.0 ± 44.3†
Bac	10^5	<15.0	7.9 ± 1.2	8.2 ± 4.7	12.0 ± 4.6
HS/bac	10^3	75.1 ± 49.3†	96.0 ± 72.0†	117.6 ± 41.2†	101.4 ± 72.8†
Bac	10^3	<15.0	11.6 ± 8.3	13.1 ± 7.2	21.7 ± 13.5
HS	0	<15.0	16.1 ± 3.6	15.1 ± 8.4	23.3 ± 24.2
SCR	0	<15.0	<5.0	<5.0	<10.0
Control	0	<15.0	<5.0	<5.0	<10.0

*Data were expressed as mean ± standard deviation.

†Significantly higher than the level of each bac, HS, SCR, and control (P < 0.05).

Figure 1. DTH induction after S. sanguis infection. DTH response of the HS/bac group is significantly higher than that of each bac group and controls (P <.05). HS/bac(7), HS/bac(5), Hs/bac(3): inoculum size of S. sanguis (10^7, 10^5, and 10^3 CFU/mouse, respectively). SCR, scraping. Data indicate mean ± standard deviation.

Figure 2A

Figure 2B

Figure 2C

Figure 2. IgG antibody responses against synthetic peptides. (A) Antibody response against hsp 336–351. (B) Antibody response against Brn-3b 11–25. (C) Antibody response against Bes-1 229–

Figure 2. IgG antibody responses against synthetic peptides. (A) Antibody response against hsp 336–351. (B) Antibody response against Brn-3b 117–129. (C) Antibody response against Bes-1,229–243. HS/bac(7), HS/bac(5), HS/bac(3): inoculum of S. sanguis (10^7, 10^5, and 10^3 CFU/mouse, respectively). SCR, scraping. Data indicate mean ± standard deviation. Experimental groups are significantly higher than that of controls.

Figure 3. Mild iridocyclitis after inoculation with S. sanguis (10^5 CFU/mouse) and heat treatment. Infiltration of polymorphonuclear leukocytes is seen in the lesion (x800).

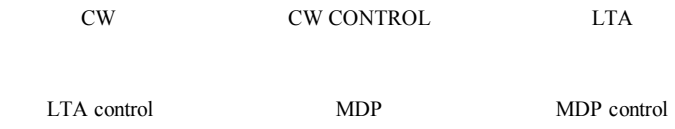

Figure 4. Histopathologic findings after intraocular injection of CW, LTA, and MDP. Injection of these materials caused influx inflammatory cells (polymorphonuclear cells) and fibrin accumulation.

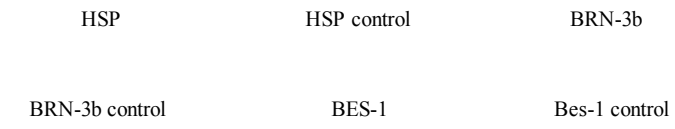

Figure 5. Histopathologic findings after intraocular injection of hsp 336–351 (HSP), Brn-3b, and Bes-1. Brn-3b caused influx inflammatory cells (polymorphonuclear cells) and fibrin accumulation but hsp 336-351did not.