Staphylococcal Enterotoxin-Activated Spleen Cells Passively Transfer Diabetes in BB/Wor Rat

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BB/Wor rats develop spontaneous autoimmune diabetes similar to human insulin-dependent diabetes mellitus. A T-cell–mediated pathogenesis for BB/Wor diabetes is indicated because disease is prevented by neonatal or adult thymectomy and treatment of diabetes-prone rats with monoclonal antibodies directed against CD5 or CD8 T-cell surface markers. Disease can be adoptively transferred with injections of concanavalin A–activated spleen cells from either acutely diabetic or RT6.1 T-cell–depleted diabetes-resistant BB/Wor rats. We used microbial superantigens to stimulate spleen cells from RT6.1 T-cell–depleted diabetes-resistant rats and demonstrated that such cells activated with staphylococcal enterotoxins (SEs) can also transfer diabetes. The diabetogenic effector T cells are readily activated by SEA, SEC3, and SEE, whereas SEB- and SEC2-activated cells are far less effective in the adoptive transfer of diabetes. These results demonstrate that microbial superantigens are capable of activating self-reactive and diabetes-inducing T cells in vitro in the BB/Wor rat. Ubiquitous microorganisms may be the environmental trigger for autoimmunity in susceptible individuals. *Diabetes* 41:527–32, 1992

The inbred BB/Wor diabetes-prone (DP) family lines have a high rate of spontaneous diabetes (60–80%). Conversely, the BB/Wor diabetes-resistant (DR) family line (derived from the same progenitors as the DP line and inbred for >30 generations for resistance to diabetes) rarely (<1%) develops spontaneous diabetes. However, DR rats harbor silent diabetogenic effector cells. DR rats injected with cyclophosphamide (1) or subjected to low-dose irradiation (2) frequently become diabetic. In vivo depletion of DR rats with a monoclonal antibody (MoAb) directed against the RT6.1 T cell alloantigen (expressed on 60% of peripheral rat T cells), may also induce diabetes (3). RT6.1 T-cell depletion results in the acquisition of diabetes transfer activity by concanavalin A (ConA)-activated RT6.1-depleted DR spleen cells (presumably by removal of regulatory T cells that prevent diabetes in DR rats (3). RT6.1-depleted DR rats were used for these studies because DP rats are profoundly lymphopenic and DP T cells give poor in vitro proliferative responses to most T-cell stimuli (4). In contrast, DR rats are nonlymphopenic and DR T cells respond readily to T-cell–dependent mitogens and the staphylococcal enterotoxins (SEs).

The *Staphylococcus aureus* toxins or SEs are pathogenetic in humans and animals. These proteins bind to human and mouse MHC proteins (5–7) and form a molecular complex with specificity for different V_β elements of the α/β T-cell receptor (TCR) for antigen (7). The composite result of this multimeric interaction is the activation of discrete sets of V_β-bearing T cells. SE-induced activation results in both proliferation and lymphokine secretion by many T cells (8). It has been suggested that the onset of autoimmune disease may be related to the initial activation of quiescent self-reactive T cells by SEs or other environmental antigens with similarly powerful T-cell stimulatory properties (9). We tested this hypothesis by asking whether SEs would activate otherwise silent BB/Wor diabetes-inducing T cells in vitro.

The adoptive transfer of BB rat diabetes requires that spleen cells first be activated in vitro by the polyclonal T-cell mitogen ConA (10). Therefore, we hypothesized that one or more of the SEs would be similarly capable of activating the diabetogenic effector T cells, possibly in a V_β-selective fashion. We report here that there is a differential activation of diabetes-inducing T cells by the SEs and that this activation seems to be dependent on

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Received for publication 6 September 1991 and accepted in revised form 18 December 1991.

prior in vivo depletion of RT6.1$^+$ regulatory T cells. SE-activated spleen cells from intact (nondepleted) DR rats do not adoptively transfer diabetes. Because freshly prepared (noncultured) spleen cells from RT6.1 T-cell–depleted DR rats are also not competent in the adoptive transfer assay, the combined data support the postulated role for SEs (or other microbial superantigens) in the triggering of quiescent self-reactive and diabetes-inducing DR T cells.

RESEARCH DESIGN AND METHODS

All BB/Wor rats in this study were raised at the Univ. of Massachusetts Medical School. DP rats raised in a specific pathogen–free (SPF) environment had an incidence of spontaneous diabetes of 50–80% between 60 and 120 days old, with a mean age at onset of 91 days. In the course of these experiments, the BB/Wor colony was cesarean-derived and maintained under viral antibody–free (VAF) conditions. In the VAF colony, the mean age at onset for diabetes shifted from 91 to 80 days, and the incidence of spontaneous diabetes increased to 75–95%. The incidence of spontaneous disease in DR rats was <1% for SPF and 0 for the VAF colony (11).

Tissue culture medium. RPMI-1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 25 ng/ml fungizone, 10 mM HEPES, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, and 5% heat-inactivated fetal calf serum was used for all tissue culture work.

RT6.1 depletion protocol. Female DR rats at 30 days of age were injected 5 times/wk with 2 ml i.p. DS4.23 (anti-RT6.1) MoAb tissue culture supernatant for 4–5 wk. Among the RT6.1-depleted DR rats, 8 of 195 became diabetic during the depletion regimen and were not used as cell donors. The DS4.23 cell line was obtained from D.L. Greiner (Univ. of Connecticut Health Science Center, Storrs).

Proliferation assay. Single-cell splenocyte populations were prepared from nondiabetic RT6-depleted DR rats and plated in RPMI-1640 with 5% heat-inactivated fetal calf serum at 6×10^5 cells/well in 96-well flat-bottomed trays (Costar, Cambridge, MA) at final vol of 0.2 ml. After 48 h, cultures were labeled with 1 μCi [^{3}H]thymidine (Amersham, Aylesbury, UK), and cells were collected 16 h later. Incorporation of [^{3}H]thymidine was measured by liquid-scintillation spectroscopy. Results are means ± SD of cpm of four replicates. ConA was obtained from ICN (Irvine, CA), SEB was from Sigma (St. Louis, MO), and all other SEs were from Toxin Technology (Madison, WI).

Flow Cytometry of SE-activated Cells. Single-cell splenocyte suspensions from RT6.1-depleted DR rats were cultured with 15 μg/ml SEC2, 5 μg/ml SEC3, and 1.75 μg/ml SEE. After 3 days, viable blasts were enriched on Ficoll-metrizoate (d = 1.077 g/ml) and grown overnight in the 48-h supernatant of ConA-activated Lewis rat splenocytes. Blasts ($1–2 \times 10^6$) were incubated with each MoAb in the form of undiluted tissue culture supernatant (each lot pretested by flow cytometry on nondiabetic rat lymphocytes to insure the presence of saturating concentrations of specific Ab). The secondary incubation used fluorescein isothiocyanate (FITC)-conjugated F(ab')$_2$ goat anti-mouse IgG (Cappel, Malvern, PA). Labeled cells were examined on a fluorescence-activated cell sorter (FACS) 440 flow cytometer (Becton Dickinson, Rutherford, NJ). MoAbs were OX8 (CD8), OX12 (rat κ–light chain), OX19 (CD5), R73 (α/β TCR), OX39 (CD25, p55, interleukin-2R), and W3/25 (CD4). Background staining was determined with mouse Ig for the primary incubation. The OX8, OX12, OX19, OX39, and W3/25 hybridoma cell lines were obtained from A.F. Williams and D.W. Mason (Oxford Univ., Oxford, UK). The R73 hybridoma cell line was obtained from T. Hunig (Univ. of Munich, Germany). Each SE was FACS analyzed on three separate occasions with the same results.

Two-color flow cytometry. Peripheral blood leukocytes (1×10^6) were incubated sequentially with OX8 (anti-CD8) MoAb tissue culture supernatant and then FITC goat anti-mouse IgG. After a blocking step with mouse IgG, the cells were incubated with biotinylated OX19 (anti-CD5) and then phycoerythrin streptavidin. Paraformaldehyde-fixed cells (0.5%) were examined on a FACS 440 and the data displayed in contour plots.

Adoptive transfer of diabetes. Single-cell splenocyte suspensions were prepared from RT6.1-depleted female DR rats and injected directly or cultured with SE or ConA at 3×10^6/ml in RPMI-1640 with 5% heat-inactivated fetal calf serum for 3 days in 5% CO_2 at 37°C. Cells were harvested, washed five times with RPMI, adjusted to contain at least 3×10^7 blasts (1–1.5 spleen equivalents), and were injected intravenously into 30- to 35-day old SPF female DP or intraperitoneally into 21- to 25-day old VAF female DP recipients. (VAF recipients were injected at a younger age than SPF recipients because of the earlier onset of diabetes in the VAF colony [80 vs. 91 days]). VAF recipients were injected intraperitoneally rather than intravenously because of their smaller size. The route of injection has no effect on the efficacy of transfer. Female rats were used because pilot studies with SEB demonstrated that female rats became diabetic at a higher incidence than male rats. Recipients of fresh spleen cells were given one-spleen equivalent of cells. Diabetes was diagnosed on the basis of glycosuria (Testape, Eli Lilly, Indianapolis, IN) and a blood glucose ≥13.8 mM (≥250 mg/dl). Testing of rats for adoptive diabetes was performed 3 times/wk by people with no knowledge of the experimental protocol. SPF rats were recipients of SEA-, SEB-, SEC3-, and ConA-stimulated cells. VAF rats were recipients of freshly prepared SEC2-, SEE-, and ConA-stimulated cells. A successful transfer was considered to be the onset of diabetes by 60 days in SPF recipients or 50 days in VAF recipients. In the case of DP recipients, onset of diabetes by 50 (VAF) or 60 (SPF) days of age was considered evidence of adoptive transfer because <0.6% of rats in these age groups become spontaneously diabetic. A cohort of age- and sex-matched DP rats was given no cells and monitored identically for spontaneous diabetes.

Propagation of SEE-stimulated short-term cell lines. Spleen cells from RT6.1-depleted DR or acutely diabetic DP rats were cultured at 6×10^6/ml in RPMI-1640, 2%

TABLE 1
Stimulation of BB/WOR RT6.1 T-cell–depleted diabetes-resistant (DR) splenocytes by staphylococcal enterotoxins (SEs)

Stimulant	Proliferation (cpm × 10^{-3})
Medium (μg/ml)	24.0 ± 2.8
SEA	
1.25	86.9 ± 12.8
2.5	81.9 ± 10.7
SEB	
2.5	77.8 ± 21.8
5	104.1 ± 19.7
SEC2	
2.5	58.8 ± 3.5
5	46.1 ± 5.7
SEC3	
2.5	128.5 ± 8.4
5	96.9 ± 9.8
SEE	
2.5	89.9 ± 20.2
5	52.4 ± 4.8
Concanavalin A	
2.5	56.8 ± 14.0
5	54.7 ± 4.5

Values are means ± SD. RT6.1 T-cell–depleted DR splenocytes were cultured with SEs for 3 days (see METHODS).

fresh autologous rat serum, and 1.5 μg/ml SEE. (The DP splenocytes were 1st cultured for 2 h in RPMI-1640, 10% fetal calf serum at 37°C, and nonadherent cells were then cultured with SEE.) At the end of 3 days, viable blast cells were enriched on Ficoll-metrizoate (d = 1.077) gradients and recultured at 10^5/ml in RPMI-1640, 10% fetal calf serum, and 5–10% Lewis rat ConA supernatant. When the rate of division slowed, cells were restimulated at 2×10^5/ml in RPMI-1640, 2% rat serum, and 1.25–2 μg/ml SEE. Irradiated (3000 R) DR or nonadherent DP spleen cells (1.5×10^6/ml) served as antigen-presenting cells. Blast cells were harvested at 72 h, washed, and injected intraperitoneally into 21- to 25-day old VAF DP recipients.

Morphological studies. Diabetic recipients of SE-stimulated cells were killed on the day of detection. Pancreases were fixed in Bouin's solution and the hemotoxylin-eosin–stained paraffin sections were examined (A.A.L.) for the presence of lymphocytic insulitis and end-stage islets without knowledge of the experimental protocol or physiological status of the rat.

RESULTS

BB/Wor splenocytes proliferate in response to SEs. The SEs are powerful T-cell stimulants in mice (12) and humans (13). Table 1 shows that SEs also stimulate a strong primary in vitro proliferative response by RT6.1-depleted BB/Wor DR splenocytes as well as, or often better than the equivalent concentrations of the polyclonal T-cell mitogen, ConA. Concentrations of each SE used in the transfer of diabetes were capable of promoting vigorous DNA synthesis by RT6.1 T-cell–depleted DR spleen cells. Culture conditions were identical for both the proliferation assays in Table 1 and the adoptive transfer studies in Tables 2–4, thus allowing a direct comparison of levels of DNA synthesis versus SE-induced activation of diabetes-inducing T cells.

TABLE 2
Adoptive transfer of diabetes by staphylococcal enterotoxin (SE)-activated RT6.1 T-cell–depleted diabetes-resistant spleen cells

Mean SE	Concentration (μg/ml)	Incidence (*n*)	Mean age at onset (days)	Mean blood glucose (mg/dl)
SEA	0.250	0 of 8		
SEA	1.25	6 of 8	54.5	339
SEA	2.5	0 of 10		
SEB	1.25	1 of 5	60	
SEB	2.5	0 of 7		
SEB	5	2 of 6	57	
SEB	10	2 of 4	58	
SEB	15	0 of 5		
SEC2	2.5	0 of 5		
SEC2	5	2 of 7	45	
SEC2	10	2 of 7	49	
SEC2	15	4 of 10	46	461
SEC3	1.25	0 of 6		
SEC3	2.5	7 of 7	57	504
SEC3	5	1 of 4	60	
SEE	1.25	7 of 8	37	368
SEE	5	8 of 8	40.5	362
Concanavalin A (ConA)	4	4 of 8	55	415
Fresh spleen cells		0 of 17		
No cells		1 of 57	58	

Recipients of ConA- or SE-activated cells were injected i.p. or i.v. with at least 3×10^7 blast cells. Recipients of fresh spleen cells were given 1-spleen equivalent of cells.

SEs activate BB/Wor T- and B-cells. RT6.1 T-cell–depleted DR spleen cells were activated in vitro by each of the SEs, the blast transformed cells enriched by density gradient centrifugation, grown overnight in T-cell growth factor–containing medium, and then analyzed for cell surface markers by single-color flow cytometry. Fig. 1 illustrates that SE-activated cells bear surface antigens for the following rat T-cell markers: α/β TCR; CD4 (helper/inducer T cells); CD8 (cytotoxic/suppressor T cells), and CD25 (p55 interleukin-2R). That these are activated T cells is demonstrated by the fact that they express high

TABLE 3
Cumulative frequencies of adoptive diabetes in diabetes-prone recipients of staphylococcal enterotoxin (SE)-activated or fresh RT6.1 T-cell–depleted diabetes-resistant spleen cells

	Diabetogenic concentration (μg/ml)	Frequency (%)	Mean age at onset (days)
SEA	1.25	75	54.5
SEB	5–10	40	57.5
SEC2	5–15	33.3	46
SEC3	2.5	100	57
SEE	1.25–5.00	93.7	39
Concanavalin A	4	50	55
Fresh spleen cells		0	
No cells		1.75	58

Cumulative frequencies derived from data in Table 2.

TABLE 4
Adoptive transfer of diabetes by nondepleted diabetes resistant spleen cells

Staphylococcal enterotoxin (SE)	Concentration (μg/ml)	Incidence (*n*)
SEA	1.25	0 of 10
SEB	2.5	0 of 8
SEC3	5	0 of 5

Recipients of SE-activated cells were injected with at least 3×10^7 blast cells.

levels of CD25, which is not expressed on resting cells. Approximately half of the SE-stimulated cells are of the T-cell lineage as determined by staining for α/β TCR, CD2, or CD5 (data not shown). SE-activated and blast-transformed cells (25–35%) express surface Ig (determined by staining for the rat κ–light chain) and/or OX33 (14), the B-cell–specific isoform of the CD45 leukocyte common antigen (data not shown), and are thus B cells.

SEs stimulate adoptive transfer of diabetes. The SEs that we tested demonstrated differential activations of the diabetogenic T cells contained within the RT6.1 T-cell–depleted DR splenocyte population (Tables 2 and 3). SEA, SEC3, and SEE were considerably more effective than an optimal dose of ConA in the adoptive transfer of diabetes, with transfer rates of 75–100%. On the other hand, SEB and SEC2 were less effective than ConA in diabetes induction, with transfer rates of ≤40%. The ability of a particular concentration of an SE to stimulate diabetogenic effector cells does not seem to be related to its ability to induce DNA synthesis (1.25 μg/ml SEA [86,960 cpm, stimulation index = 3.6] vs. 2.5 μg/ml SEA [81,950 cpm, stimulation index = 3.4]; Table 1. SEA-stimulated cells (1.25 μg/ml) adoptively transferred disease in 6 of 8 recipients, whereas 2.5 μg/ml SEA-stimulated cells transferred diabetes in 0 of 10 recipients. Finally, freshly prepared RT6.1 T-cell–depleted spleen cells (not activated in culture) were not capable of adoptively transferring diabetes (0 of 17).

SE-stimulated adoptive transfer of diabetes is immunologically mediated. Spontaneous diabetes in the BB/Wor rat (and human insulin-dependent diabetic patients) is characterized at the onset of hyperglycemia by a mononuclear cell infiltrate (insulitis) within the pancreatic islets of Langerhans. The BB/Wor infiltrate is comprised of macrophages, $CD5^+$ T cells, $CD8^+$ T cells, and, at the very latest stage of the process, B cells (15,16). Subsequent to the onset of clinical diabetes, insulitis rapidly subsides and only end-stage islets are present. End-stage islets are depleted of β-cells by the autoimmune attack and contain only glucagon-, somatostatin-, and pancreatic polypeptide–synthesizing endocrine cells. All diabetic recipients of SE-stimulated cells exhibited destructive insulitis and the presence of end-stage islets. Normal or uninfiltrated islets were absent (data not shown).

Diabetic recipients of SE-activated cells have increased numbers of T cells. Two-color flow cytometry of peripheral blood lymphocytes from acutely diabetic recipients of SE-stimulated RT6-depleted DR splenocytes revealed the presence of surviving DR T cells. DP rats are profoundly lymphopenic, with abnormally low numbers of $CD4^+$ ($CD8^-/CD5^+$) T cells (17). DP rats are also almost entirely deficient in $CD8^+/CD5^+$ T cells (17). The presence of surviving DR T cells in DP recipients was manifest as a significant increase in the percentages of both $CD8^+$ and $CD4^+$ T cells. The mean percentage of $CD8^+/CD5^+$ T cells was 8.25 ($n = 25$), and the mean percentage of $CD4^+$ ($CD8^-/CD5^+$) T cells was 21.6 ($n = 25$). In contrast, age- and sex-matched uninjected DP rats had a mean percentage of 0.78 ($n = 17$) $CD8^+/CD5^+$ and 5.85 ($n = 17$) $CD4^+$ T cells ($P < 0.0001$ by Student's *t* test). Because the SEs stimulate both $CD4^+$ and $CD8^+$ T cells (Fig. 1), it is not possible to identify the phenotype of the diabetes-inducing T cells from these data.

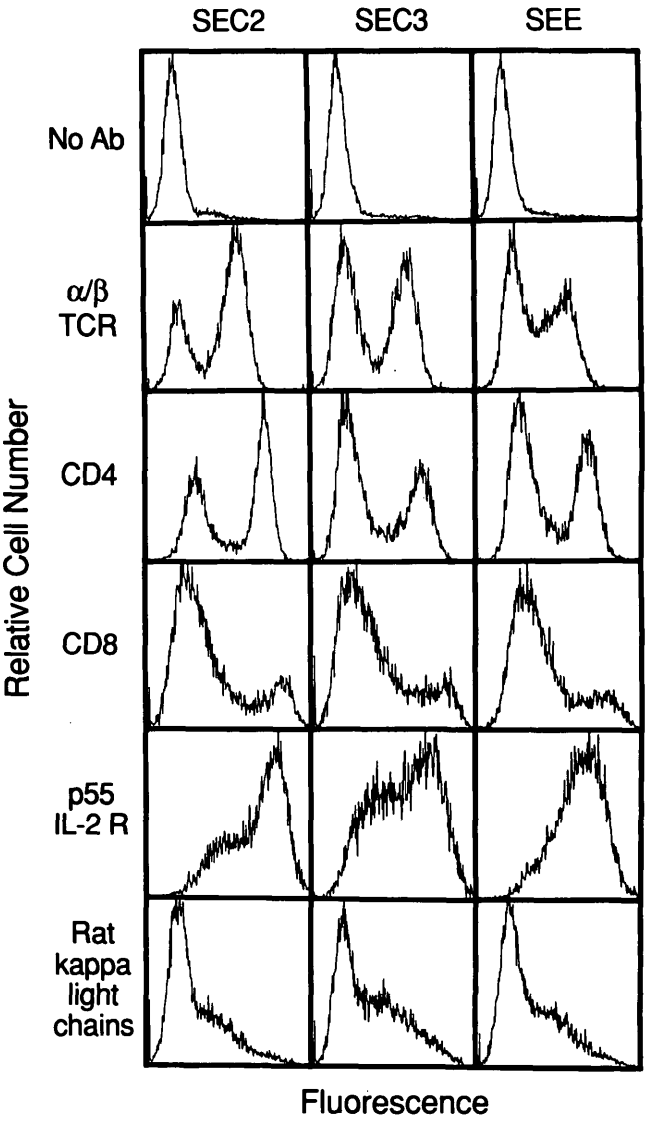

FIG. 1. Expression of lymphocyte cell surface markers on staphylococcal enterotoxin (SE)-activated RT6.1-depleted diabetes-resistant (DR) lymphoblasts. RT6.1 T-cell–depleted DR splenocytes were cultured for 3 days with 15 μg/ml SEC2, 5 μg/ml SEC3, or 1.75 μg/ml SEE. Ficoll-metrizoate gradient-enriched blast cells were grown overnight in the 48-h supernatant of concanavalin A–activated Lewis rat splenocytes and then prepared for single-color flow cytometry.

TABLE 5
Adoptive transfer of diabetes by staphylococcal enterotoxin E (SEE)-stimulated short-term cell lines

Source	*n* Cells × 10^6	Incidence (*n*)	Mean age at onset (days)
Acute diabetic diabetes-prone rat	35	3 of 3	39
RT6-depleted diabetes-resistant rat	35–40	3 of 5, 4 of 4	38

Recipients of SEE-activated cells were 21- to 25-day-old diabetes prone viral antibody–free rats.

SE-activated cells from intact (non–RT6.1-depleted) DR donors do not transfer diabetes. To control for the possibility that any SE-stimulated DR spleen cell could adoptively transfer diabetes by releasing disease-activating lymphokines into the circulation of recipient rats, identical experiments were performed by culturing spleen cells from non–RT6.1-depleted DR rats with SEs (Table 4). In these experiments, 0 of 8 recipients of 2.5 μg/ml SEB-stimulated cells, 0 of 10 recipients of 1.25 μg/ml SEA-stimulated cells, and 0 of 5 recipients of 5 μg/ml SEC3-stimulated cells developed diabetes. SE-induced DNA synthesis and blast transformation were equivalent in RT6.1-depleted and nondepleted DR spleen cell populations (data not shown). These results suggest that SE-induced activation of diabetogenic effector T cells may occur only in the absence of regulatory T cells (as has been shown previously for ConA [3]) and that the transferred diabetes may result from specific ligation of effector cell α/β TCR.

SEE-stimulated short-term cell lines adoptively transfer diabetes. Attempts were made to establish effector T-cell lines with SEE, the most potent of the SEs in the adoptive transfer assay. RT6-depleted DR splenocytes or acutely diabetic DP spleen cells were stimulated in vitro with SEE. After 3 days, the blast-transformed cells were enriched on density gradients (to separate out the SEE nonreactive cells) and recultured in Lewis rat spleen cell ConA supernatant (as a source of T-cell growth factors). When the rate of division slowed, cells were restimulated with SEE and irradiated splenocytes (as antigen-presenting cells). Three days later, cells were harvested, and 35–40 × 10^6 blast cells were injected intraperitoneally into 21- to 25-day-old DP recipients. Table 5 shows that these cells, from both RT6-depleted DR and acutely diabetic DP donors, were capable of transferring adoptive diabetes within an average of 2 wk postinjection. All diabetic recipients of SEE-stimulated short-term cell lines exhibited destructive lymphocytic insulitis and typical end-stage islets. Although the transferred cells have not been proven to be T cells, they were derived with a standard protocol for the establishment of rat T-cell lines (18). Although the cells could not subsequently be maintained in culture, efforts are under way to establish long-term diabetes-inducing T-cell lines that are SE-specific.

DISCUSSION

SE-stimulated BB/Wor DR T cells readily transfer diabetes, whereas fresh (uncultured) spleen cells are not competent in the transfer of disease. These results support the hypothesis that environmental pathogens and/or their extracellular products may set autoimmunity in motion by stimulating sufficient numbers of quiescent self-reactive T cells. Other microbial antigens have been described that also stimulate large numbers of T cells. A mitogen derived from *Mycoplasma arthritidis* selectively stimulates $V_\beta6^+$ and $V_\beta8^+$ T cells in the mouse (19). *Mycoplasma arthritidis* also induces chronic arthritis in mice. Similarly, streptococcal M protein, the major virulence factor for *group A Streptococci*, is a V_β-specific stimulant of nonimmune human T cells (20). Infection of susceptible individuals with *group A Streptococci* can lead to several autoimmune disorders. The specific contribution of these microbial superantigens to postinfectious autoimmune sequelae is unknown.

Staphylococcus aureus is a normal component of the indigenous human microflora, and many children and adults have detectable levels of circulating antibodies to the SEs (21–23), indicating past or chronic exposure. Additionally, approximately one-third of all *Staphylococcus aureus* clinical isolates are enterotoxin producers (24). The initiation of autoimmunity may thus coincide with subclinical infections by enterotoxin-producing strains in susceptible individuals. Susceptibility may be determined by MHC class II allelism. It has been reported that there is a differential presentation of SEs to human T-cell hybridomas that is dependent on the HLA-DR allele expressed on the antigen-presenting cell (25). Additionally, the tendency of autoimmune diseases to spontaneous exacerbations and remissions may be due, in some cases, to intermittent activation of effector cells by such subclinical encounters with SEs or other microbial superantigens.

SE-induced activation of T cells leads to proliferation and lymphokine secretion both in vitro and in vivo (8,26). Autoimmunity could thus result from several different mechanisms. Direct stimulation of self-reactive T cells might lead to organ-specific damage by either cell-mediated cytolysis or by the secretion of toxic cytokines in high local concentrations. Alternatively, the superantigen-activated T cells might release quantities of lymphokines sufficient to nonspecifically stimulate otherwise quiescent autoimmune T cells. The superantigenic capabilities of other bacterial or viral gene products and their role in autoimmunity remain unassessed. That SEs appear to activate rat B cells suggests that antibody-mediated autoimmunity may also be turned on by bacterial or viral superantigens.

BB/Wor autoimmune diabetes is T-cell mediated; it is prevented by adult or neonatal thymectomy (27,28) and by the in vivo treatment of diabetes-prone rats with MoAbs to CD5 or CD8 T-cell-surface markers (29). The T-cell nature of this autoimmune disorder suggested that TCR V_β-selective reagents would be useful in both activating and identifying the diabetogenic effector T cells. We report a differential activation of diabetes-inducing T cells by the SEs: SEA, SEC3, and SEE are considerably

more effective than the optimal dose of ConA in the adoptive transfer of diabetes, whereas SEB and SEC2 are far less potent activators of the diabetogenic effector cells. Nevertheless, it is clear that each of the tested SEs is competent, even if marginally, in promoting the adoptive transfer of diabetes. Because each SE stimulates multiple and overlapping V_β-specific T-cell subsets in mice and humans (12,13), our data do not allow any conclusions regarding TCR V_β gene usage in this model of autoimmunity. However, unless each of the SEs used in the adoptive transfer assay stimulates a single, shared V_β-specific TCR in the BB/Wor DR rat, then TCR V_β gene usage in this disease model would not be restricted.

Intact (non-RT6.1 T-cell–depleted) DR spleen cells activated by SEs did not transfer diabetes in our experiments. Apparently, the RT6.1$^+$ regulatory T cells do not permit SE-induced activation of diabetogenic effector cells. The presence of silent diabetogenic effector T cells in intact DR rats demonstrates that not all autoreactive T cells are removed by clonal deletion in the thymus (30,31). Instead, they are under peripheral regulatory control. Transient disturbances in such peripheral regulatory mechanisms may allow microbial superantigens to turn on autoimmune processes.

We have recently reported that environmental virus infection by Kilham's rat virus (KRV) can initiate immune insulitis and diabetes among DR rats (32). KRV-induced diabetes may result from stimulation of β-cell cytotoxic T cells by a viral gene product. That KRV-induced diabetes occurs in the absence of detectable direct viral-induced β-cell cytopathology (before onset of insulitis) and that immune suppression prevents diabetes in KRV-infected DR rats (unpublished observations), suggests that KRV may function as a viral superantigen in the initiation of autoimmunity.

Our experiments with RT6.1-depleted DR spleen cells demonstrate that microbial superantigens activate self-reactive and diabetes-inducing T cells in vitro. The possibility arises that viral superantigens initiate DR diabetes in vivo by similarly stimulating β-cell–destructive T cells.

REFERENCES

1. Like AA, Weringer EJ, Holdash A, McGill P, Atkinson D, Rossini AA: Adoptive transfer of autoimmune diabetes mellitus in Biobreeding/Worcester (BB/W) inbred and hybrid rats. *J Immunol* 134:1583–87, 1985
2. Handler ES, Mordes JP, McKeever U, Nakamura N, Bernhard J, Greiner DL, Rossini AA: Effects of irradiation on diabetes in the BB/Wor rat. *Autoimmunity* 4:21–30, 1989
3. Greiner DL, Mordes JP, Handler ES, Angelillo M, Nakamura N, Rossini AA: Depletion of RT6.1$^+$ T lymphocytes induces diabetes in resistant Biobreeding/Worcester (BB/W) rats. *J Exp Med* 166:461–75, 1987
4. Elder ME, Maclaren NK: Identification of profound peripheral T lymphocyte immunodeficiencies in the spontaneously diabetic BB rat. *J Immunol* 130:1723–31, 1983
5. Fraser JD: High-affinity binding of staphylococcal enterotoxins A and B to HLA-DR. *Nature* (Lond) 339:221–23, 1989
6. Mollick JA, Cook RG, Rich RR: Class II MHC molecules are specific receptors for staphylococcal enterotoxin A. *Science* 244:817–19, 1989
7. Marrack P, Kappler J: The staphylococcal enterotoxins and their relatives. *Science* 248:705–11, 1990
8. Carlsson R, Sjogren HO: Kinetics of IL-2 and interferon-gamma production, expression of IL-2 receptors, and cell proliferation in human mononuclear cells exposed to staphylococcal enterotoxin A. *Cell Immunol* 96:175–83, 1985
9. White J, Herman A, Pullen A, Kubo R, Kappler J, Marrack P: The V beta–specific superantigen staphylococcal enterotoxic B: stimulation of mature T cells and clonal deletion in neonatal mice. *Cell* 56:27–35, 1989
10. Koevary S, Rossini AA, Stoller W, Chick WL, Williams RM: Passive transfer of diabetes in the BB/W rat. *Science* 220:727–28, 1983
11. Like AA, Guberski DL, Butler L: Influence of environmental viral agents on frequency and tempo of diabetes mellitus in BB/Wor rats. *Diabetes* 40:259–62, 1991
12. Callahan J, Herman A, Kappler J, Marrack P: Stimulation of B10.BR T cells with superantigenic staphylococcal toxins. *J Immunol* 144: 2473–79, 1990
13. Choi Y, Kotzin B, Herron L, Callahan J, Manack P, Kappler J: Interaction of Staphylococcal aureus toxin "superantigens" with human T cells. *Proc Natl Acad Sci USA* 86:8941–45, 1989
14. Woollett G, Barclay A, Puklavec M, Williams AF: Molecular and antigenic heterogeneity of the rat leukocyte-common antigen from thymocytes and T and B lymphocytes. *Eur J Immunol* 15:168–73, 1985
15. Hanenberg H, Kolb-Bachofen V, Kantwerk-Funke G, Kolb H: Macrophage infiltration precedes and is a prerequisite for lymphocytic insulitis in pancreatic islets of pre-diabetic BB rats. *Diabetologia* 32:126–34, 1989
16. Lee KU, Kim MK, Amano K, Pak CY, Jaworski MA, Mehta JG, Yoon JW: Preferential infiltration of macrophages during early stages of insulitis in diabetes-prone BB rats. *Diabetes* 37:1053–58, 1988
17. Woda BA, Like AA, Padden C, McFadden ML: Deficiency of phenotypic cytotoxic-suppressor T lymphocytes in the BB/W rat. *J Immunol* 136:856–59, 1986
18. Ben-Nun A, Wekerle H, Cohen I: Rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. *Eur J Immunol* 11:195–99, 1981
19. Cole B, Kartchner D, Wells D: Stimulation of mouse lymphocytes by a mitogen derived from Mycoplasma arthritidis (MAM). *J Immunol* 144:425–31, 1990
20. Tomai M, Kotb M, Majumdar G, Beachey E: Superantigenicity of streptococcal M protein. *J Exp Med* 72:359–62, 1990
21. Reiser R, Jacobsen J, Kasworm E, Bergdoll MS: Staphylococcal enterotoxin antibodies in pediatric patients from Utah. *J Infect Dis* 158:1105–108, 1988
22. Jozefczyk Z, Robbins R, Spitz J, Bergdoll MS: Antibodies to staphylococcal enterotoxin in laboratory personnel. *J Clin Microbiol* 11: 438–39, 1980
23. Jozefczyk Z: Specific human antibodies to enterotoxins A, B and C1 of Staphylococcus: their increased synthesis in staphylococcal infection. *J Infect Dis* 130:1–7, 1974
24. Joklik W, Willett H, Amos DB, Wilfert CM: *Zinsser Microbiology.* Norwalk, CT, Appleton & Lange, 1988, p. 350
25. Herman A, Croteau G, Sekaly R, Kappler J, Marrack J: HLA-DR alleles differ in their ability to present staphylococcal enterotoxins to T cells. *J Exp Med* 172:709–17, 1990
26. Newell K, Ellernhorn J, Bruce D, Bluestone J: In-vivo T cell activation by staphylococcal enterotoxin B prevents outgrowth of a malignant tumor. *Proc Natl Acad Sci USA* 88:1074–78, 1991
27. Like AA, Kislauskis E, Williams RM, Rossini A: Neonatal thymectomy prevents spontaneous diabetes mellitus in the BB/Wor rat. *Science* 216:644–46, 1982
28. Like AA: Depletion of RT6.1$^+$ T lymphocytes alone is insufficient to induce diabetes in diabetes-resistant BB/Wor rats. *Am J Pathol* 136:565–74, 1990
29. Like AA, Biron CA, Weringer EJ, Byman K, Sroczynski E, Guberski DL: Prevention of diabetes in Biobreeding/Worcester rats with monoclonal antibodies that recognize T lymphocytes or natural killer cells. *J Exp Med* 164:1145–59, 1986
30. MacDonald HR: T cell receptor V beta use predicts reactivity and tolerance to Mls a–encoded antigens. *Nature* (Lond) 332:40–45, 1988
31. Kappler J, Staerz U, White J, Marrack P: Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. *Nature* (Lond) 332:35–40, 1988
32. Guberski DL, Thomas VA, Shek WR, Like AA, Handler ES, Rossini AA, Wallace JE, Welsh RM: Induction of type I diabetes by Kilham's rat virus in diabetes-resistant BB/Wor rats. *Science* 254:1010–13, 1991.