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Blockade of Late Stages of Autoimmune Diabetes by Inhibition of the Receptor for Advanced Glycation End Products

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Ligation of the receptor for advanced glycation end products (RAGE) occurs during inflammation. Engagement of RAGE results in enhanced expression of addressins and it is therefore, not surprising that previous studies have shown a role of RAGE/ligand interactions in immune responses including cell/cell contact but the role of RAGE in spontaneous autoimmunity has not been clearly defined. To study the role of RAGE/ligand interactions in autoimmune diabetes, we tested the ability of soluble RAGE, a scavenger of RAGE ligands, in late stages of diabetes development in the NOD mouse-disease transfused with diabeticogenic T cells and recurrent disease in NOD/scid recipients of syngeneic islet transplants. RAGE expression was detected on CD4+, CD8+, and B cells from diabetic mice and transferred to NOD/scid recipients. RAGE and its ligand, S100B, were found in the islets of NOD/scid mice that developed diabetes. Treatment of recipient NOD/scid mice with soluble RAGE prevented transfer of diabetes and delayed recurrent disease in syngeneic islet transplants. RAGE blockade was associated with increased expression of IL-10 and TGF-β in the islets from protected mice. RAGE blockade reduced the transfer of disease with enriched T cells, but had no effect when diabetes was transferred with the activated CD4+ T cell clone, BDC2.5. We conclude that RAGE/ligand interactions are involved in the differentiation of T cells to a mature pathogenic phenotype during the late stages of the development of diabetes. The Journal of Immunology, 2004, 173: 1399–1405.

Advanced glycation end products (AGEs)3 are formed by the nonenzymatic glycation and oxidation of proteins and/or lipids, normally during aging, and at an accelerated rate in diabetes (1–3). AGEs such as carboxy(methyl)lysine, pentosidine, and other AGEs have been identified in the tissues from patients and animals with diabetes. It is thought that direct modification of cellular structure and function may account for the toxicity of AGEs, but in addition, these molecules bind to a cell surface receptor (RAGE), a multiligand member of the Ig superfamily, and induce cell activation (4–6). In addition to the AGES, the natural ligands for RAGE include members of the S100/calgranulin family and amphoterin (7, 8). RAGE is expressed at low levels on a variety of cell types, but its expression is increased by cellular activation that occurs during inflammation (5, 9). The cellular activation that is seen may be attributable to activation of a signal transduction cascade involving P21Wos, ERK-1 and -2 kinases, Sin3-associated protein-JNK, and Cdc42-Rac (9–13).

More recent studies have described a role of RAGE/ligand interactions in immune and inflammatory responses. Engagement of RAGE by AGEs results in enhanced expression of addressins such as VCAM-1 (3, 14, 15). Zeng et al. (28) have recently shown that blockade of RAGE with a soluble form of the receptor (soluble RAGE (sRAGE)) prevented liver reperfusion injury and was associated with decreased levels of TNF-α at the inflammatory sites. RAGE-deficient mice have a normal phenotype, and using these mice, Chavakis et al. (16) identified RAGE as the counterreceptor for Mac-1, and the interaction of RAGE with Mac-1 was augmented by the proinflammatory RAGE-ligand, S100B. They found decreased migration of neutrophils to the peritoneum in thioglycollate-induced peritonitis in RAGE−/− mice. At sites of immune responses, such as in the spinal cord of mice with experimental autoimmune encephalomyelitis (EAE), increased expression of RAGE and its ligand, S100B, has been observed (17). Cellular activation and secretion of cytokines and growth factors were seen following RAGE activation in this model. Blockade of RAGE activation with sRAGE was shown to prevent delayed type sensitivity reactions (5). Yan et al. (17) recently reported that RAGE-ligand interactions modulated encephalitogenic CD4+ T cells that induced EAE.

These previous observations indicate that RAGE/ligand interactions may play a role in regulating immune responses, but its role in autoimmune diabetes has not previously been tested. Because of the increased levels of RAGE ligands and AGEs in diabetes, this axis has particular importance. Therefore, we have studied the role of RAGE/ligand interactions in autoimmune diabetes in the NOD mouse. Many nonspecific interventions are able to prevent diabetes in the NOD mouse when initiated at the earliest stages of islet inflammation (18). These approaches may not have application to later stages of the disease when the immune process is more fully matured and diversified. Because the majority of individuals who develop type I diabetes mellitus cannot be identified before the initiation of autoimmunity, these approaches are of uncertain clinical value. Therefore, we chose to test whether RAGE/ligand interactions are involved in later disease processes, and studied the
role of RAGE during recurrent diabetes following adoptive transfer of diabeticogenic spleen cells and after transplantation of syngeneic islets into diabetic NOD mice. These two models are closest to clinical situations in which immune interventions are likely to be used. We have found that RAGE/ligand interactions are involved in late event of diabetes in the NOD mouse and that blockade of this interaction alters the phenotype of the autoimmune response. Our studies suggest that the effect is on differentiation of T cells in the presence of Ag rather than on mature effector cells because the blockade prevented diabetes transferred with T cells but not with monoclonal effector T cells. This common link between the toxic effects of glycation end products and immune responses suggests a mechanism whereby control of glucose may improve β cell function.

Materials and Methods

Mice and reagents

Both female NOD/LtJ mice and female NOD/scid mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained under pathogen-free conditions at our facility. BDC2.5/NOD male mice obtained from Stony Brook (Southampton, NY) were bred for two generations to female NOD/scid mice to get BDC2.5/NOD-scid mice, which develop diabetes within 2–2.5 wk after birth. Young NOD/scid (6- to 8-wk-old) mice served as recipients in all cell transfers. BDC2.5/NOD-scid mice served as donors in the BDC2.5 cell transfer, spontaneous autoimmune diabetic NOD/LtJ mice (12–25 wk old) served as donors in all the other cell transfers. NOD mice were screened for glycosuria three times a week beginning at 12 wk of age, and considered diabetic when two blood glucose levels were >250 mg/dl on two occasions. Young, prediabetic NOD/LtJ (6- to 7-wk-old) mice served as islet donors and spontaneous autoimmune diabetic NOD mice (12–25 wk old) served as recipients in the islet transplantation experiment. Blood glucose levels were measured with the Glucometer Elite XL (Bayer, Elkhart, IN). Spontaneous autoimmune diabetes was diagnosed by blood glucose levels >250 mg/ml on 2 consecutive days.

Soluble mouse RAGE

Murine sRAGE was expressed using a baculovirus system and purified to homogeneity (19), and dissolved in sterile, endotoxin-free PBS. sRAGE was administered by i.p. route (50 μg per day), and control animals received equal volumes of sterile, endotoxin-free PBS. The monoclonal anti-RAGE Abs, 1B11H and 3A6D5, were generated in our laboratories (5).

Adoptive transfer of diabetes

Splenocytes adoptive transfer. Single-cell suspensions were prepared from spleens of diabetic NOD/LtJ females by passage through preseparation filters (Miltenyi Biotec, Auburn, CA). Erythrocytes were lysed, and the nucleated cells were washed, counted, and resuspended in PBS (103 per 200 μl). Cells were injected i.v. via the lateral tail vein into recipient NOD/scid mice (200 μl per mouse). sRAGE was administered daily beginning on the day of cell transfer until diabetes developed or until all of the control animals, and those treated with murine serum albumin (MSA), developed diabetes.

Enriched T cell adoptive transfer. Splenocytes were isolated as described above. B cells were removed using anti-CD19 microbeads (Miltenyi Biotec) using the manufacturer’s protocol. The cells were injected i.v. via the lateral tail vein into recipient NOD/scid mice. sRAGE was administered daily beginning on the day of cell transfer until diabetes developed or until all of the control animals, and those treated with mouse serum albumin (MSA), developed diabetes.

BD2C.5 adoptive transfer. Splenocytes were obtained as described above from spleens of diabetic NOD/BD2C.5 mice, 2–3 wk of age. NOD/scid mice received a transfer of 0.6–4 × 106 cells. sRAGE was administered and diabetes was diagnosed as described above.

Islet isolation

Mice were anesthetized with ketamine chloride (Fort Dodge Animal Health, Fort Dodge, IA). After intradural injection of 3 ml of cold HBSS (Life Technologies, Grand Island, NY) containing 1.5 mg/ml collagenase P (Roche Diagnostics, Branchburg, NJ), pancreata were surgically procured and digested at 37°C for 20 min. Islets were washed by HBSS and purified by discontinuous gradient centrifugation using polysucrose 400 (Mediatech, Herndon, VA) with four different densities (26, 23, 20, and 11%). The tissue at the interface of the 20 and 25% layers were collected, washed, and resuspended in HBSS. Individual islets, free of attached acinar, vascular, and ductal tissues, were handpicked under an inverted microscope, yielding highly purified islets for transplantation or RNA extraction.

Syngeneic islet transplantation

Diabetic NOD/LtJ mice received islet grafts within 5 days of the diagnosis of diabetes. Five hundred to 600 freshly isolated islets (~550 islet equivalents) from prediabetic NOD/LtJ were picked up with an infusion set and transplanted into the subcapsular space of the right kidney (20, 21). Islet graft function was monitored by serial blood glucose measurements daily for the first 2 wk after islet transplantation, followed by every other day thereafter. Reversal of diabetes was defined as blood glucose <200 mg/dl on two consecutive measurements. Graft loss was determined when blood glucose exceeded 250 mg/dl on two measurements.

Flow cytometry

The following mAbs were used for T cell analysis: PE-conjugated rat anti-CD4, APC-conjugated rat anti-mouse CD4, PE-conjugated rat anti-mouse CD8a, PerCP-conjugated rat anti-mouse CD8a, and PE-conjugated rat anti-mouse B220 (all from BD Pharmingen, San Diego, CA). RAGE-specific mouse mAb, 3A6D5, was prepared and characterized, then conjugated with FITC using a FITC-labeling kit (Molecular Probes, Eugene, OR). Flow cytometry was performed on a FACScan (BD Biosciences, San Jose, CA). Data were collected on 0.5 × 106 cells and reanalyzed using CellQuest software (BD Biosciences).

Histology and immunohistochemistry

Frozen tissue sections were stained with rat anti-mouse CD4, rat anti-mouse CD8a (both from BD Pharmingen), RAGE-specific rabbit IgG, S100B-specific rabbit antiserum (1/100 dilution; Sigma-Aldrich, St. Louis, MO), TNF-α, and IL-1β, followed by biotinylated goat anti-rat IgG (BD Pharmingen) or biotinylated goat anti-rabbit IgG (BD Pharmingen), prediluted SAv-HRP (BD Pharmingen), and DAB substrate kit for peroxidase (Vector Laboratories, Burlingame, CA) were used to produce a brown or gray-black color. All sections were counterstained with Mayer’s hematoxylin solution.

Insulitis was determined from H&E-stained pancreas sections. The severity of insulitis was scored in blinded fashion using the following criteria: 0, no cellular infiltrates; 1, scattered cells infiltrating the islet; 2, numerous cells infiltrating the islet but with retention of islet architecture; 3, extensive cellular infiltrate into the islet with loss of islet architecture; peri-insulitis, cells surround but do not infiltrate the islets.

Analysis of cytokine gene expression by real-time PCR

Islets were isolated as described above, total RNA was extracted from 500 islets with TRIzol reagent (Life Technologies) and cDNA was prepared according to the manufacturer’s protocol (Life Technologies). For every reaction set, one RNA sample was performed without Superscript II RT (RT minus reaction, Invitrogen Life Technologies, Carlsbad, CA) to provide a negative control in subsequent PCR.

Real-time PCR was performed using an ABI-Prism 7700 sequence detector and Sequence Detection Software (Applied Biosystems, Foster City, CA). Products were detected using SYBR Green (Molecular Probes) or gene-specific oligonucleotide molecular beacon reactions. Reactions (50 μl volume) contained 2 U of AmpliTaq Gold (Applied Biosystems), 20 pmol of each primer, 10 pmol of molecular beacon, and 60 nM carboxy-X-rhodamine as a passive reference (22). Product copy number was determined by comparison to standard curves generated using ampiclon-containing plasmids as template. For analysis, the copy number was divided by the copy number of hypoxanthine phosphoribosyltransferase in the sample to correct for the amount of starting material.

Statistical analysis

The time to diabetes was compared between sRAGE and control groups by a Mantel-Cox test of a Kaplan-Meier life table analysis. The effects of sRAGE treatment on the survival of islet grafts was tested with a Mann-Whitney U test of the time to development of diabetes after transplantation.

Results

Expression of RAGE and RAGE ligands in the insulitis and on immune cells that transfer diabetes to NOD/scid mice

To determine whether RAGE and its ligand, S100B, are expressed in the insulitis in NOD diabetes, we studied the expression of these
molecules in a model of adoptive transfer of diabetes using splenocytes from diabetic NOD mice infused into immune-deficient NOD/scid mice. Expression of RAGE and S100B were studied by immunohistochemistry 6 wk after transfer, a time at which the mice develop diabetes (Fig. 1). RAGE and S100B ligand were not detected in the islets from NOD/scid mice before cell transfer. However, RAGE and S100B were expressed on islet cells with an inflammatory infiltrate in sections of pancreata from diabetic NOD/scid mice that had received a transfer of splenocytes. Both endocrine and nonendocrine cells expressed both molecules.

To determine whether RAGE is expressed on immune cells, we studied the expression of RAGE on T and other cells from the spleen of diabetic NOD mice by flow cytometry using mAb 3A6D5. Splenocytes were harvested from diabetic NOD mice, and without further activation in vitro, stained with anti-RAGE Ab and other cell surface markers (Fig. 2). Electronic gates were placed around CD4^+^, CD8^+^, or B220^+^ cells. In a representative experiment, RAGE was detected on 37% of CD4^+^, 40% of CD8^+^, and 46% of B cells that were transferred to NOD/scid recipients.

**Blockade of RAGE activation prevents transfer of diabetes into NOD/scid mice and recurrent autoimmune diabetes in recipients of islet transplants**

We used two models of recurrent disease to evaluate the role of RAGE/ligand interactions in autoimmune diabetes: transfer of diabetes into NOD/scid mice with spleen cells from a diabetic NOD donor and recurrent diabetes in diabetic NOD mice that receive an islet transplant.

NOD/scid mice that received a transfer of 10^7^ splenocytes from a diabetic NOD donor were treated with either sRAGE (50 μg per day), a decoy for RAGE ligands, or MSA (19). Treatment with sRAGE significantly reduced the rate of transfer of diabetes (p < 0.0001; Fig. 3). By day 36 after transfer, 22 of 24 (92%) control animals, but only 2 of 25 (10%) mice treated with sRAGE, were diabetic.

Insulitis was reduced by treatment with sRAGE (Table I). In the control animals, 69% of the islets had grade 2 or 3 insulitis, whereas in islets from sRAGE-treated mice, only 23% of islets showed this severity (Table I). However, there was an increase in the number of islets that showed noninfiltrative “peri-insulitis”, generally considered a nonpathogenic lesion in sRAGE-treated animals. The change in insulitis was not accompanied by a change in the distribution of T cell subsets within the inflammatory lesions that were seen in the islets. The relative proportion of CD4^+^ and CD8^+^ T cells was similar in the lesions from control and sRAGE-treated mice (Fig. 4). Thus, lymphocytes appeared to migrate to islets in the sRAGE-treated mice but assumed a nonpathogenic configuration.

RAGE blockade also prevented recurrent diabetes when syngeneic islet cells were transferred into diabetic, immune-competent NOD host (Fig. 5). Diabetic NOD mice received an islet graft of 500 islets underneath the kidney capsule followed by treatment with sRAGE or mouse albumin. Disease recurrence occurred in the control animals within 30 days of transplant, whereas grafts were not rejected in the sRAGE-treated mice until 44 days after transplant with an average delay in graft rejection of 25 ± 3 days (p < 0.001).

**Treatment with sRAGE reduces the expression of inflammatory mediators and alters the phenotype of islet infiltrating T cells after transfer of diabetogenic spleen cells**

Our studies of the sRAGE recipients of transferred cells indicated that T cells were activated in recipients, and that T cells had infiltrated the islets but did not cause recurrent disease. To determine how sRAGE treatment had altered the inflammatory process, we studied the expression of inflammatory mediators and cytokines in the islets following adoptive transfer of splenocytes with and without sRAGE treatment. The expression of TNF-α and IL-1β, inflammatory cytokines thought to be directly involved in β cell destruction, was studied by immunohistochemistry. Treatment with sRAGE decreased the expression of IL-1β and TNF-α (Fig. 6). In control animals, 39 ± 3% and 34 ± 4% of the islet area stained for IL-1β and TNF-α, whereas in the sRAGE-treated animals, 14 ± 4% and 3.0 ± 0.4% of the area showed expression of these mediators (both p < 0.001).

The basis for this change in inflammatory mediators in the presence of RAGE blockade was not clear from our studies of T cell activation in vivo because they had indicated that T cells had migrated to the islets after transfer. To determine the effects of sRAGE treatment on the islet-infiltrating T cells, islets were isolated from NOD/scid mice 14 days after they received a transfer of splenocytes, and quantitative expression of cytokine mRNA by the cells infiltrating the islets was measured by RT-PCR (Fig. 7). The levels of expression of IFN-γ or IL-4 in islets from control and sRAGE-treated mice were similar. However, the expression of...
IL-10 was increased nearly 10-fold in sRAGE-treated mice and there was also a significant increase in the expression of TGF-β in these islets (both p < 0.05).

sRAGE blockade affects a heterogeneous population of T cells, but not fully activated T cells

These findings suggested an effect of sRAGE treatment on the differentiation of T cells into a pathologic phenotype. However, in both adoptive transfer and islet transplantation, those cells that cause disease likely represent a small proportion of the T cell repertoire and our studies of cytokine production suggested that, as a result of the production of IL-10 and TGF-β, RAGE blockade may even lead to the induction of cytokines associated with regulatory cells or phenomenon. Moreover, from our initial flow studies, RAGE was expressed on both splenic T and B cells. Therefore, to determine whether non-T cells, or even a heterogeneous T cell repertoire, were needed for the sRAGE effects, we studied the effects of RAGE blockade in NOD/scid mice that received a transfer of enriched T cells or a monoclonal population of activated T cells from BDC2.5 transgenic NOD mice. The BDC2.5 T cells were isolated from diabetic mice in which the transgene was expressed on a NOD/scid background. In these mice, diabetes occurs by 3 wk of age.

Similar to our findings with whole spleen cells, sRAGE treatment prevented diabetes when T cells were transferred, suggesting that the non-T cells that were present in the splenocyte transfer were not needed for protection (Fig. 8A). In contrast, there was no effect of sRAGE on diabetes transferred with BDC2.5 cells (Fig. 8B). Diabetes occurred shortly after the transfer of cells in sRAGE-treated and control animals. Even when the number of BDC2.5 cells transferred was reduced to as few as 0.6 × 10⁶ per mouse, the time of diabetes onset in the recipient NOD/scid mice was the same in sRAGE-treated mice as in the control mice (9 days for both). In addition, like our findings with whole splenocytes, dilution of CFSE in labeled BDC2.5 T cells was not reduced by sRAGE treatment suggesting that Ag recognition and T cell proliferation was not affected (data not shown).

Discussion

We have studied the role of RAGE/ligand interactions in the late stages of autoimmune diabetes in the NOD mouse. There are many interventions that can prevent diabetes when they are introduced at the early stages of the disease in this model, but relatively few that can prevent the disease in its later stages (18). In addition, the model systems we used for study are closest to the clinical situations in which blockade of RAGE might be considered. We have shown that RAGE is involved in the inflammatory responses of diabetes that occurs following the adoptive transfer of diabetes into immune-deficient NOD/scid mice or recurrent diabetes in NOD mice following islet transplantation. RAGE was expressed on both T and non-T cells that were adoptively transferred. Blockade of RAGE was associated with nonpathologic inflammatory lesions in recipient NOD/scid mice and a greater proportion of noninfiltrating T cells. Inflammatory mediators that are thought to be involved in diabetes were reduced in the islets of the treated mice. Moreover, there was an increase in the expression of IL-10 and TGF-β in the islets of sRAGE-treated recipients of transferred cells. The significance of the increased expression of IL-10 in this setting is not known at this point—it may be secondary to the thwarted immune response or may have a more primary role in prevention of recurrent disease, possibly even through induction of regulatory T cells. The effects of the RAGE blockade involved the transferred T cells directly and did not require other non-T cells because the RAGE blockade prevented transfer of diabetes with T cells. However, sRAGE was completely ineffective in preventing diabetes transferred with a monoclonal population of highly activated CD4+ T cells. This suggests that RAGE blockade affects an earlier stage in T cell development that is associated with differentiation of cells into mature effectors. The final stages of β cell destruction that is conducted by activated effector cells is not affected by RAGE blockade.

Our studies of RAGE expression on transferred splenocytes showed expression of RAGE on T, as well as B, and other non-T cells and our histologic studies show RAGE and S100B expression on endocrine cells as well within the inflamed islets. Thus, a heterogeneous population of cells express RAGE during inflammation. Previous studies by Yan et al. (17) have emphasized the importance of RAGE interactions on pathologic CD4+ T cells because a CD4+ T cell clone that caused EAE could be inhibited by blocking RAGE. Recently, studies by Chavakis et al. (16) using a RAGE-deficient mouse have provided evidence for interaction between RAGE expressed on T cells and Mac-1 expressed on macrophages. In these studies, activated human neutrophils were found to adhere to immobilized RAGE or RAGE-transfected K562 cells, and the adherence could be inhibited by anti-Mac-1 Ab. However, non-T cells also express RAGE and T cells express RAGE ligands including S100B. Therefore, the direction of the RAGE ligand interaction is still not resolved but may be addressed with selective
elimination of the ligand. In this regard, it was recently shown that a dominant-negative RAGE molecule, expressed on CD4\(^+\) T cells, prevented induction of experimental allergic encephalomyelitis with myelin basic protein (MBP) suggesting that RAGE expression on T cells is necessary (17).

Our findings indicate that while RAGE blockade affects late events in type I diabetes, not all T cells are equally affected. A heterogeneous population of transferred splenic T cells was blocked from causing disease, but fully activated CD4\(^+\) effector cells were not. Clearly, the transferred population of splenocytes included cells that were capable of causing diabetes—they had done so in the host before transfer. However, in the presence of RAGE blockade, it is likely that an increased proportion of the previously uncommitted cells present in the population of transferred T cells differentiate into IL-10 and TGF-\(\beta\) producers than without RAGE blockade. The basis for this is not clear from our studies, but the findings from other model systems that have shown a direct interaction between RAGE and \(\beta_2\) integrins raise the possibility that signals delivered to T cells in the presence of RAGE blockade are inadequate to cause differentiation into Th1 cells that cause disease. The function of the IL-10\(^+\) and/or TGF-\(\beta\)^+ cells cannot be determined from our studies—these cytokines are immunoregulatory and therefore, these cells may mediate immune regulation at least locally (23, 24). However, data from IL-10\(^{-/-}\) mice that develop chronic inflammatory colitis, would suggest that at least IL-10 is not required for the immune modulatory effects of RAGE blockade since treatment of these mice with sRAGE prevented disease (5). Thus, while the increased expression of IL-10 and TGF-\(\beta\) suggests that immune regulation may be involved in the effects of RAGE blockade, this point is not clearly resolved from our findings.

Nonetheless, it is clear from our studies, that the effects of RAGE blockade depended on the presence of these cells when the repertoire of transferred T cells was limited to a differentiated CD4\(^+\) diabeticogenic T cell clone, RAGE blockade had no effect. The failure to block the transfer was not likely to be due to the large number of pathogenic T cells that were transferred because even when the number of transferred cells was reduced to 600,000 per recipient, an effect of RAGE blockade was not seen. Interestingly, a similar dependence on a diverse T cell repertoire has been observed in the prevention of diabetes by heterotopic expression of IL-4 by islet cells (25). In this model of diabetes on the NOD background, rat insulin promoter-IL-4 transgenic mice were protected from disease unless a second transgene that limited the repertoire to BDC2.5\(^+\) T cells was also expressed.

Initially, our findings appear to differ from a recent report in which RAGE blockade was found to prevent EAE (17). In the EAE model, blockade of RAGE prevented the transfer of disease by an encephalitogenic T cell clone as well as in a transgenic mouse expressing a pathogenic TCR on an immune-deficient background. The reported studies suggested that RAGE was involved with the migration of CD4\(^+\) T cells to the inflammatory site. However, in the EAE studies, the inhibition of the CD4\(^+\) T cell clone...
was seen when the clone was transferred into an immune-competent host. In these animals, cells that had the potential to differentiate into the immune modulatory cells in a manner analogous to the transfer of enriched T lymphocytes that we observed. In addition, in the studies of mice expressing the MBP-specific encephalitogenic CD4+ T cell clone on an immune-deficient induction of disease still required immunization with MBP and the RAGE blockade was instituted before the disease still required immunization with MBP and the RAGE controls (p = 0.05).

Our studies also suggest a novel mechanism that may account for the observed improvement in insulin secretion that is seen with intensive metabolic control of type I diabetes. This phenomenon has been observed in several clinical studies including the diabetes control and complications trial in which stimulated levels of C-peptide, even after 4 years, were found to be significantly higher in diabetic mice (p < 0.05), but not after transfer of BDC2.5 T cells.

In summary, we have shown that RAGE/ligand interactions are involved in the pathogenesis of recurrent diabetes following adoptive transfer of splenocytes into NOD/scid recipients and following transplantation of syngeneic islets into diabetic NOD recipients. RAGE is expressed on immune and nonimmune cells, and therefore, the direction of the RAGE/ligand interactions are still not resolved. However, RAGE/ligand interactions are clearly involved in the differentiation of T cells into pathogenic and nonpathogenic effector cells which occurs when heterogeneous populations of T cells are transferred. When the repertoire is limited to differentiators, there is no effect. These studies suggest that RAGE blockade may represent a feasible approach to treatment particularly to individuals in whom RAGE ligands are increased as occurs in diabetes.

References


