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Long-Term Reversal of Established Autoimmunity upon Transient Blockade of the LFA-1/Intercellular Adhesion Molecule-1 Pathway

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Transgenic models and administration of mAbs directed against the LFA-1/intercellular adhesion molecule 1 (ICAM-1) pathway have shown that these costimulatory molecules play a key role in generating effector cells mediating inflammatory responses. In this report, durable remission of recent diabetes in nonobese diabetic (NOD) mice was induced by transient expression of an immunoadhesin gene encoding the soluble form of ICAM-1 (sICAM-1/Ig). A single i.v. injection of an adenovirus vector encoding the immunoadhesin gene led to 70% diabetes remission as opposed to 0% in mice injected with a control adenovirus vector. Despite the rapid decline of sICAM-1/Ig serum levels, diabetes remission remained stable in 50% of NOD mice for >6 mo. sICAM-1/Ig expression also led to long-term protection against diabetes in prediabetic NOD mice. sICAM-1/Ig in vitro induced an agonistic effect of T cell activation in a TCR-transgenic model, increasing T cell proliferation and IL-2 secretion. Importantly, protected mice were not immunosuppressed because they rejected skin allografts normally and developed immunity against the adenovirus vector. Rather, sICAM-1/Ig induced active tolerance, as assessed by the persistence of diabetogenic T cells in protected mice and the reversal of protection by immunosuppression with cyclophosphamide. The Journal of Immunology, 2002, 168: 3641–3648.

A dhesion molecules are critical for the homing, cell migration, and delivery of costimulatory signals of immunocompetent cells (1). The LFA-1 is a member of the leukocyte integrin family. LFA-1 is important in mediating cellular interactions in the immune system such as cytotoxic T cells and NK cell-mediated cytotoxicity, helper T lymphocyte responses, and leukocyte adhesion (2). In a recent study, Camacho et al. (3) demonstrated that intercellular adhesion molecule 1 (ICAM-1), the major counterreceptor of LFA-1, plays a key role in generating effector T cells which induce autodestructive responses in a transgenic model of autoimmune diabetes. In this study, we describe a novel approach that transiently targets the LFA-1/ICAM-1 pathway and durably restores self-tolerance in vivo in a context of overt autoimmunity. Our strategy was based on an adenovirus (Ad)-mediated gene transfer of soluble ICAM-1 (sICAM-1) to achieve high, but transient, circulating levels of the expressed protein. To that end, we first engineered a chimeric gene encoding a protein in which the extracellular domain of the membrane ICAM-1 is covalently linked to the C\textsubscript{H}2 and C\textsubscript{H}3 domains of a mouse IgG1 heavy chain (4). We then used Ad-mediated transfer of the chimeric gene in nonobese diabetic (NOD) mice that spontaneously develop a T cell-mediated autoimmune diabetes that closely resembles the human disease (5). In this study, we demonstrated that in contrast to most immunointerventions including the administration of Abs to LFA-1, Ad-mediated gene transfer of the sICAM-1/Ig immunoadhesin has the remarkable capacity to reverse established autoimmune diabetes in a durable fashion, without affecting the capacity of the host to react to other unrelated foreign tissular or nontissular Ags.

Materials and Methods

sICAM-1/Ig adenoviral vector (Ad.sICAM-1/Ig)

Construction of the cDNA encoding murine sICAM-1 and an IgG1-Fc fragment was similar to TNFR-Ig heavy chain construction (4). Briefly, the C\textsubscript{H}2-C\textsubscript{H}3 domains of murine IgG1 cDNA and the extracellular domain of murine ICAM-1 cDNA were amplified using PCR. PCR primers were as follows: 5'-ggatccggggaaccatGTTCAGGTACACATTCCT-3' (corresponding to the 1453–1436 bp of the murine sICAM-1 cDNA and the Chol site), 5'-ctgttcgccggtcgtgggtggtcaggtgtccattgga-3' (corresponding to the thrombin cleavage site and the 5' end of the IgG1 moiety), 5'-attaagcattctagaTCATTTACCAGGAGAGTG-3' (corresponding to the 5' end of the IgG1 and a XbaI site). sICAM-1 cDNA joins IgG1-Fc cDNA through the thrombin cleavage site. Both fragments were cloned in TA cloning plasmid (Invitrogen, San Diego, CA) through a common BamHI site, digested with Chol and Xhol sites, and ligated into Ad shuttle plasmid. To obtain a replication-deficient recombinant Ad vector, shuttle plasmid was cotransfected into 293 cells (CRL 1573; American Type Culture Collection, Manassas, VA) with pJMi7 plasmid containing the Ad type 5 genome (Microbix Biosystems, Toronto, Canada). The replication-deficient Ad vector Ad.null contains an expression cassette including an Ad promoter with no exogenous gene and was used as a control Ad vector (6). Ad vectors were propagated, purified, and titered as previously described (6).

Production and purification of sICAM-1/Ig protein

HeLa cells (CCL 2; American Type Culture Collection) were incubated with Ad.sICAM-1/Ig at 40 PFU/cell. sICAM-1/Ig was purified from the
cell supernatant using a goat anti-mouse IgG1-Fc (Sigma-Aldrich, St. Louis, MO). Purity of sICAM-1/Ig was confirmed by silver staining after 5% SDS-PAGE. Purified sICAM-1/Ig was quantified using the Lowry test. Western blot analyses were performed using an Ab anti-mouse ICAM-1 (3E2; BD Pharmingen, San Diego, CA) after SDS-PAGE in nonreducing conditions of mice sera. sICAM-1/Ig serum levels were quantified by ELISA using two ICAM-1 Abs: a rat Ab anti-mouse ICAM-1 (KAT-1; R&D Systems, Minneapolis, MN), and a bioitin-conjugated hamster Ab anti-mouse ICAM-1 (3E2; BD Pharmingen). Purified sICAM-1/Ig was used as a standard.

**Mice histopathology**

NOD (K<sup>−</sup>, I-A<sup>−</sup>, D<sup>−</sup>), NODscid, and congenic C57BL/6-H2<sup>−</sup> mice (12 backcrosses) have been bred in our animal facility for many years under specific pathogen-free conditions (Institut National de la Santé et de la Recherche Médicale, Unite 25, Paris, France). NOD females developed insulits by 4 wk of age and spontaneous diabetes appeared by 14 wk of age (95% incidence at 40 wk). The transgenic NOD BDC2.5 and NOD C57BL/6 mice were kindly provided by D. Mathis and C. Benoist (Harvard Medical School, Boston, MA) (7); the mice used in the present experiments were backcrossed 12 times onto the NOD background. By crossing these two latter strains, we obtained NOD BDC2.5.C57BL/6 mice in which all T cells express a rearranged TCR<sup>β</sup> from a CD4<sup>+</sup> diabetogenic T cell clone. These mice are devoid of immunoregulatory T cells; 85% of them develop overt diabetes by 4–6 wk of age. In some experiments, C57BL/6 mice transgenic for expression of a TCR of known specificity recognizing FCC88-104 in association with I<sup>β</sup>2 MHC class II molecules (AND TCR), and that were rag-2 deficient (8), were used. AND TCR chain transgenic mice on a Rag-2 deficient background have a pure population of naïve T cells as no rearrangement of endogenous β or α TCR chains could occur in these mice. These mice will be referred to as AND TCR chain transgenic mice. Mice were monitored for glycosuria and fasting glycemia with colormetric strips (Glukotest and Glucorend; Boehringer Mannheim, Indianapolis, IN). Diabetes was defined when a fasting glycemia >3 G/L was determined on two consecutive occasions. Recombinant Ad vectors were administered in vivo in NOD mice using a single i.v. injection of 2.5 × 10<sup>9</sup> PFU. Complete remission was defined as the disappearance of glycosuria and a return to normal glycemia. For histopathology, paraffin-embedded sections from pancreata were stained with H&E, and the severity of insulitis was assessed by using the following criteria: grade 0 = normal islets; grade 1 = focal or peripheral insulitis (lymphocytes around the islet); and grade 2 = invasive destructive insulitis.

**Adhesion test**

Purified T cell populations were obtained from spleen and lymph nodes after B cell depletion. Transfused cell supernatant was incubated at 2°C after B cell depletion. Purified T lymphocytes were stained with metabolic fluorochrome 2'-7'-bis-(2-carboxyethyl)-5-(-6)-carboxyfluorescin (Calbiochem, La Jolla, CA), treated with PMA and incubated at 4°C for 8–10 h. Lymph node T cell or splenic T cell supernatant using a goat anti-mouse IgG1-Fc (Sigma-Aldrich, St. Louis, MO) was incubated at 2°C after B cell depletion. Puriﬁed T cell populations were obtained from spleen and lymph nodes

**Results**

**In vitro and in vivo production of sICAM-1/Ig**

To overexpress murine sICAM-1 in a dimeric form, an adenovirus vector encoding an immunoadhesin with murine sICAM-1 and IgG1-Fc cDNAs (Ad.sICAM-1/Ig) was constructed. HeLa cells were then incubated with Ad.sICAM-1/Ig and maintained in serum-free medium for 3 days. Protein afﬁliation chromatography with an Ab against the murine IgG1-Fc domain was used to purify sICAM-1/Ig proteins from cell supernatants. SDS-PAGE analysis showed that sICAM-1/Ig was a dimer, migrating at 200 kDa when nonreducing conditions were used (Fig. 1A, lane 1) and 100 kDa when reduced (Fig. 1A, lane 2). These sizes were in agreement with those predicted for sICAM-1/Ig (11).

Recombinant Ad vectors were administered in vivo in NOD mice using a single i.v. injection of 2.5 × 10<sup>9</sup> PFU. This dose was chosen after the in vivo dose escalating assays revealed high serum levels of circulating sICAM-1/Ig without liver inﬂammation (not shown). By comparison to puriﬁed sICAM-1/Ig (Fig. 1B, lane 1), Western blot analysis with an anti-mouse ICAM-1 Ab detected circulating sICAM-1/Ig in mice sera 3 days after i.v. injection of the Ad.sICAM-1/Ig vector (Fig. 1B, lane 2). In contrast, no circulating sICAM-1/Ig was detected in mice sera after injection of the control Ad vector Ad.null (Fig. 1B, lane 3).

To demonstrate that sICAM-1/Ig could bind its homologous ligand LFA-1, murine T lymphocyte adhesion to immobilized sICAM-1/Ig was evaluated (Fig. 1C). Activated T cells did not adhere substantially to empty wells in the absence (Fig. 1C, left empty bar; value ± SE) or presence of ICAM-1 (Fig. 1C, middle empty bar; value ± SE) or LFA-1 Abs (Fig. 1C, right empty bar; value ± SE). In contrast, activated T cells bound to wells coated with sICAM-1/Ig (Fig. 1C, left crossed bar; value ± SE). Adhesion was totally inhibited by preincubation with an anti-LFA-1 Ab (Fig. 1C, left crossed bar; value ± SE vs the value in the middle bar + SE, p < 0.001), and partially inhibited by the anti-ICAM-1 Ab (Fig. 1C, left crossed bar; value ± SE vs the value in the right crossed bar + SE, p < 0.001). This confirms that T cells adhered to sICAM-1/Ig-coated wells through LFA-1/ICAM-1 binding.

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**Adenoviral-mediated sICAM-1/Ig gene transfer induced long-term remission of established diabetes**

Overt diabetes in NOD females was identified by screening for glycosuria twice a week. When glycosuric, mice were tested for hyperglycemia. Mice showing fasting glycemia >3 G/L on two
The vast majority of the islets were either intact (60%, grade 1). In contrast, in mice that were protected from disease by the injection of Ad.sICAM-1/Ig, the vast majority of islets were either normal (48%, grade 0) or had an infiltrate that remained confined to the periphery of the islets (noninvasive peri-insulitis) (16%, grade 1).

Remission of established diabetes (disappearance of glycosuria and a return to normal glycemia) was observed in 70% of Ad.sICAM-1/Ig-treated mice 10 days after Ad injection, and was still up to 50% 200 days after Ad injection (Fig. 2; $p < 0.0001$). In contrast, no remission was observed in any mouse treated with Ad.null. Instead, these mice died rapidly. Histologic examination of pancreata was performed 200 days after i.v. injection of Ad.sICAM-1/Ig or Ad.null. Complete remission was defined as the disappearance of glycosuria and a return to normal glycemia. Concurrently, sICAM-1/Ig serum levels were quantified by ELISA.

To examine whether long-lasting protection from diabetes could be induced by transient expression of sICAM-1/Ig well after induction of insulitis but still at a preclinical stage, nondiabetic female NOD mice at 14 wk of age were injected i.v. with Ad.sICAM-1/Ig, Ad.null, or control buffer (Fig. 4). No statistically significant difference in the incidence of diabetes was observed over time between control and Ad.null animals, with diabetes incidence of 70 and 55%, respectively, at 40 wk of age (Fig. 4). In contrast, the transient sICAM-1/Ig expression that followed the Ad administration induced a significant and lasting protection from diabetes, as compared with control and Ad.null-injected mice, with the incidence of diabetes dropping significantly to 26% in this group ($p = 0.0006$; Fig. 4). Interestingly, there was a positive correlation between sICAM-1/Ig serum levels and the incidence of diabetes. Effective disease protection was only observed in mice showing a serum sICAM-1/Ig concentration $>10$ μg/ml ($p = 0.005$).

Histologic evaluation of pancreata was performed in protected animals at 40 wk of age and in control animals at 25 wk when the incidence of diabetes was 50% (Fig. 3B). Although in all experimental groups, insulitis was observed, the topography of the infiltration significantly differed depending on the treatment. Pancreatic islets of control mice (buffer and Ad.null-injected) which developed a high incidence of the disease showed severe invasive/destructive insulitis (68 and 57%, grade 2; Fig. 3B). In clear contrast, in mice that were protected from disease by the injection of Ad.sICAM-1/Ig, the vast majority of islets were either normal (48%, grade 0) or showed peri-insulitis (28%, grade 1; Fig. 3B).
wk after Ad.sICAM-1/Ig injection (i.e., when no more sICAM-1/Ig was detected) in Ad.sICAM-1/Ig-protected mice (not shown). Finally, to evaluate the development of immunity directed against recombinant Ad vectors, female NOD mice were injected at 12 wk of age with Ad.sICAM-1/Ig, Ad.null, or control buffer. Four weeks later, all mice were reinjected with Ad.sICAM-1/Ig. In contrast to control buffer mice, no serum sICAM-1/Ig could be detected in Ad.sICAM-1/Ig or in Ad.null-treated mice, suggesting that a strong immunity against the Ad vector has developed. To evaluate the presence of Abs against sICAM-1/Ig, we performed the T cell adhesion test in the presence of both sICAM-1/Ig and serum from mice 14–32 days after Ad.sICAM-1/Ig injection. If these mice were immunized against sICAM-1/Ig, the Abs in their serum should bind to sICAM-1/Ig and block sICAM-1/Ig adhesion, as shown in the presence of anti-ICAM-1 or anti-LFA-1 Ab (Fig. 1C). Seven sera from mice injected with Ad.null, and 20 from mice injected with Ad.sICAM-1/Ig, were evaluated. None of these sera inhibited T cell adhesion to sICAM-1/Ig, suggesting that these sera did not contain Ab against sICAM-1.

sICAM-1/Ig transient expression did not delete diabetogenic cells

To determine whether the protective effect of Ad.sICAM-1/Ig gene therapy was due to a direct effect on diabetogenic T cells, adoptive transfer studies were performed. Splenocytes from Ad.sICAM-1/Ig-treated NOD mice showing durable protection 14 wk after Ad injection were injected into NODscid recipients. In some experiments, purified CD62L<sup>+</sup> splenocytes were injected, because diabetic T splenocytes concentrate among CD62L<sup>+</sup> cells (10). Unseparated or CD62L<sup>+</sup>-purified splenocytes from Ad.sICAM-1/Ig-treated mice transferred diabetes as efficiently as splenocytes from untreated diabetic NOD mice (Fig. 5). Similarly, BDC2.5 TCR-transgenic NOD mice, which only express a rearranged TCR<sup>+</sup> from a CD4<sup>+</sup> diabetogenic T cell clone (7), were injected with Ad.sICAM-1/Ig or Ad.null. No protection against diabetes was observed in NOD BDC2.5<sup>+</sup> mice treated with Ad.sICAM-1/Ig (n = 5, Ad.sICAM-1/Ig; n = 4,
Ad.null-treated mice; diabetes incidence 80% in each group at day 40, 100% at day 80), confirming that sICAM-1/Ig gene transfer did not alter diabetogenic cells. Finally, cyclophosphamide (200 mg/kg) was administered to mice that had been injected with Ad.sICAM-1/Ig at 14 wk of age and did not develop diabetes at 30–40 wk of age, and to a control group of nondiabetic female NOD mice at 9–10 wk of age. Cyclophosphamide injection induced diabetes in 7 of 10 Ad.sICAM-1/Ig-treated mice and in 6 of 8 mice in the control group, confirming that diabetogenic cells were still present in Ad.sICAM-1/Ig-treated mice despite protection from diabetes.

sICAM-1/Ig expression did not alter lymphocyte homing to the pancreas

Histopathological analysis of pancreata from protected NOD mice (Fig. 3) argued against an exclusive effect of sICAM-1/Ig gene transfer on lymphocyte homing because the insulitis, although significantly decreased as compared with controls, was not totally prevented. To further evaluate whether the LFA-1/ICAM-1 pathway plays a role for the migration of islet-specific mononuclear cells, we also attempted to block the adoptive transfer of diabetes to NODscid mice following the injection of islet-derived mononuclear cells from newly diabetic donors, by administering Ad.sICAM-1/Ig to the recipients. sICAM-1/Ig serum levels in NODscid mice reached a maximum 7 days after injection, then decreased, and remained detectable for 8 consecutive wk (Fig. 6). Injection of Ad.sICAM-1/Ig in NODscid mice 3 wk after adoptive transfer did not prevent diabetes (Fig. 6). Because under these experimental conditions, Ad.sICAM-1/Ig was injected at a time point when cells home to the pancreas to some extent and the diabetes process starts, experiments were also performed in which Ad.sICAM-1/Ig injection was injected 3 days before adoptive cell transfer. Early Ad.sICAM-1/Ig injection was also totally ineffective in preventing diabetes (diabetes incidence in Ad.sICAM-1, Ad.null, and control groups of >80% 7 wk after cell transfer, and of 100% 9 wk after cell transfer). These results suggest that Ad.sICAM-1/Ig did not induce the remission of diabetes by preventing the extravasation of autoreactive T cells to the islets.

![FIGURE 6](http://www.jimmunol.org/) Absence of effect on diabetes transfer of sICAM-1/Ig transient expression in NODscid mice. NODscid mice were transfused with spleen cells collected from untreated diabetic NOD mice. Three weeks after cell injection, mice were injected i.v. with Ad.sICAM-1/Ig (△), Ad.null (■), or control buffer (○). Animals were regularly monitored for glycosuria and glycemia. Concurrently, sICAM-1/Ig serum levels (●) were quantified by ELISA.

sICAM-1/Ig acted as an agonist of ICAM-1/LFA-1-induced costimulation

To further examine the sICAM-1/Ig effect on T cell activation, we evaluated the sICAM-1/Ig agonist or antagonist effect on ICAM-1/LFA-1 costimulation. Lymph node naive T cells from AND TCR chain transgenic mice were cocultured with APCs in the presence of varying concentrations of the specific peptide, and in the presence of sICAM-1/Ig and/or anti-LFA-1 Ab. As expected (9), the anti-LFA-1 Ab inhibited both cell proliferation, as measured by thymidine uptake (Fig. 7A), and IL-2 secretion (Fig. 7B). In marked contrast, sICAM-1/Ig induced T cell proliferation in the absence and presence of peptide at 24 h (not shown) and 48 h (Fig. 7A), and increased IL-2 secretion at 24 h (Fig. 7B), demonstrating a stimulatory effect of sICAM-1/Ig on T cell activation. The sICAM-1/Ig stimulatory effect was totally inhibited by anti-LFA-1 Ab (not shown), confirming that sICAM-1/Ig induced T cell proliferation and IL-2 production through ICAM-1/LFA-1 binding.

Discussion

Insulin-dependent diabetes mellitus develops spontaneously in NOD mice by 15 wk of age due to the selective destruction of insulin-secreting β cells by autoreactive CD4+ and CD8+ cytotoxic lymphocytes (5). Overt disease is preceded by an infiltration of the islets of Langerhans by mononuclear cells which starts by 3 wk of age and which initially remains confined to the periphery of the islets (i.e., peripheral insulitis). In quite an abrupt manner, by 12 wk of age, the infiltrate invades the islets and actively destroys β cells through mechanisms that are not fully understood (12). Our data demonstrate that a single injection of an Ad vector encoding an immunoadhesin induces long-term remission of overt diabetes in NOD mice. The Ad.sICAM-1/Ig-induced remission was durable (>6 mo) and was maintained despite the presence of peripheral, but not invasive/destructive, insulitis. These results are in clear contrast with most other immunointervention strategies described in NOD mice, which have only been shown to be effective in young animals (13, 14). In fact, only very few agents have been...
reported to be similarly effective at reversing overt autoimmunity in a durable fashion, including polyclonal anti-lymphocyte serum (15) and anti-CD3 Ab (16). These strategies induce a short-term immunosuppression associated with variable lymphopenia that precedes their tolerance-promoting effect. In marked contrast, the long-term protection observed upon the transient expression of sICAM-1/Ig was not linked to any visible modification in the distribution of the main lymphocyte subsets (not shown) or any evidence of generalized immunosuppression. The fact that skin allograft rejection was not influenced at all even in recipients showing high levels of sICAM-1/Ig stresses that the immunoadhesin is by no means an immunosuppressive drug. Finally, a second injection of Ad.sICAM-1/Ig, 4 wk after Ad.null or Ad.sICAM-1/Ig first injection, was totally inefficient in inducing detectable sICAM-1/Ig serum levels, suggesting that a neutralizing immune response to the Ad vector efficiently developed. Longer expression of sICAM-1/Ig in immunocompromised NODscid as compared with NOD mice also suggests that an immune reaction has occurred in NOD mice, leading to rapid elimination of the Ad vector. This again stresses the very low, if any, immunosuppressive capacity of the sICAM-1/Ig-expressed immunoadhesin. This is a fundamental issue because it also relates to the specificity of the observed effect. Ad.sICAM-1/Ig-treated mice were in fact unresponsive to islet Ags while they exhibited a normal response toward exogenous tissue or nonislet Ags. We also verified that no Ab against sICAM-1/Ig was raised after Ad.sICAM-1/Ig injection, suggesting that the effect of sICAM-1/Ig was not mediated by the induction of the anti-ICAM-1 Ab. As a whole, these data strongly argue for the capacity of Ad.sICAM-1/Ig to restore self-tolerance in this context of established autoimmunity.

The protection observed upon the transient expression of sICAM-1/Ig was not linked to the elimination or deletion of diabeticogenic cells, as demonstrated by the persistence of diabeticogenic cells in transfer experiments. Furthermore, the finding that a single injection of cyclophosphamide, an alkylating agent that has been shown to selectively affect T cell regulation (5), rapidly and reproducibly reversed sICAM-1/Ig-induced protection provided further support for the fact that Ad.sICAM-1/Ig-protected hosts still harbored significant proportions of diabeticogenic effectors. This sensitivity to cyclophosphamide also suggests that immunoregulatory T cells, similar to T cells that have been shown to control disease onset in prediabetic NOD mice, could still be functional in Ad.sICAM-1/Ig-protected hosts. This is further supported by the present observation that Ad.sICAM-1/Ig did not alter diabetes incidence in BDC 2.5/CAβ-/- transgenic mice that are devoid of immunoregulatory T cells and in which all T cells express a rearranged TCRαβ from a CD4+ diabeticogenic T cell clone (17). These results are reminiscent of those obtained in CD3 Ab-treated mice (16). In this model also, protected animals showed durable remission of diabetes while expressing clear signs of overt pathogenic autoreactivity. Just as presently described for sICAM-1/Ig-treated NOD mice, whole spleen cells from anti-CD3-treated NOD mice transferred disease as efficiently as splenocytes from untreated diabetic NOD.

One central question concerns the mechanism(s) allowing the metabolic reconstitution in animals presenting with overt disease as we observed in the present study. Although one cannot exclude that β cell regeneration may occur to some extent, there is compelling evidence from both the experimental and clinical field to show that at the time of diabetes onset, there is a sufficient insulin-secreting β cell mass left to allow, if the treatment is started early and if the immunological assault is efficiently controlled, to recover an adequate metabolic balance. Thus, Sreenan et al. (18) recently reported that in NOD mice at the time of overt diabetes, the residual β cell mass still represented 30% of control levels. Moreover, in these same animals insulin secretion was reduced to a greater degree than β cell mass, suggesting the presence of β cell dysfunction in addition to reduced mass. This is fully in keeping with the notion that the physical destruction of β cells is to a large extent preceded by a phase of reversible T cell-mediated inflammation that translates into a significant impairment in their capacity to release insulin in response to conventional stimulations. In fact, heavily infiltrated pancreatic islets from nondiabetic 13-wk-old female NOD mice failed to respond with insulin secretion to high glucose concentrations when examined immediately after the isolation. This inhibition of β cell function was fully reversible upon clearing of the immune cell infiltrate after 7 days of in vitro culture (19). In vivo in the mouse, agents, such as antilymphocyte serum (15), Abs to CD3 (20), and TCRαβ (21) that rapidly clear the insulitis, induce complete normalization of glycemia within no more than 24-48 h if applied within the first 7-10 days from overt diabetes onset. This effect is independent from the more durable
tolerogenic properties some of these treatments may have. Thus, at variance with CD3 Ab and antilymphocyte serum, Abs to TCRαβ do not restore self tolerance as well illustrated by the disease re-
lapse invariably observed within a few days from the end of treat-
ment (21). Similarly in the clinic, a steady and durable increase in
endogenous insulin production was observed after a combination
of insulin and cyclosporin in recently diagnosed insulin-dependent
diabetes mellitus patients (22, 23). Adult patients were enrolled
to receive cyclosporin or placebo within 1–2 mo after the first clinical
symptoms had appeared. The proportion of disease remission, as
assessed by stimulated C peptide production and the decrease of
the insulin need, was significantly higher in patients receiving the
active drug as compared with placebo.

Given the well-established role of integrins in general and
LFA-1 in particular in lymphocyte homing (24), and our data
showing that sICAM-1/Ig binds to T cell lymphocytes and there-
fore may block T cell adhesion, we explored whether the disease
remission observed upon Ad.sICAM-1/Ig administration could be
due to a blockade of the migration of fully differentiated autore-
active effectors present in the diabetic NOD host within the target
tissue. The data we obtained using diabetes transfer experiments
and Ad.sICAM-1/Ig injections are strongly against this hypothesis.
Moreover, the histopathological data recovered from NOD mice in
diabetes remission 200 days after i.v. Ad injection, as well as in
mice protected from diabetes onset, showed evidence of peripheral
insulites. These results are consistent with recent evidence demon-
strating that upon Ag priming TCR-transgenic CD4+ ICAM-1−/−
effector cells migrated in the pancreata but did not cause diabetes
(3). Another recent study in transgenic NOD ICAM-1−/− mice
showed the absence of autoimmune diabetes in these mice, con-
firming the important role of ICAM-1 in the development of au-
toimmune diabetes (25). However, in this case, no insulitis was
observed in the NOD ICAM-1−/− mice, in contrast to our results.

At the present time, it is difficult to explain the difference in our
observations and studies using anti-LFA-1 and/or anti-ICAM-1,
which have only been shown to be effective in young animals (26, 27).
In contrast to anti-LFA-1 Abs, Ad.sICAM-1/Ig did not sig-
ificantly alter the TH1/TH2 balance, as assessed in ribonuclease
27). In contrast to anti-LFA-1 Abs, Ad.sICAM-1/Ig did not sig-
ificantly delay in overt diabetes. However, when Ad vectors
were injected in overtly diabetic mice, no remission was observed
in any Ad.null mouse, suggesting that remission was not induced
by Ad injection itself. Another Ad control encoding an irrelevant
Ig heavy chain was not included in this study, because the Fc
moiety, which provides Ab-like effector functions to sICAM-1
(complement and FcR-bearing cell activation), might participate in
the therapeutic effects. The main advantage of using Ad-mediated
gene transfer of sICAM-1/Ig is that a single i.v. injection of
Ad.sICAM-1/Ig was sufficient to induce very high and sustained
levels of circulating ICAM-1. In comparison, daily injections of
recombinant sICAM-1 were necessary for 5 mo to prevent diabetes
in young NOD mice (33).

Our present data suggest the induction and/or restoration of im-
munoregulatory or dominant tolerance immune mechanisms that
closely resemble those described in young prediabetic NOD (5, 16,
34) mice. Interestingly enough, this may be achieved through
the delivery of an agent, sICAM-1/Ig, that is totally deprived of any
suppressiv activity. Apart from its obvious potential clinical ap-
lication in recently diagnosed diabetic patients, the possibility of
restoring self-tolerance in overtly autoimmune animals has funda-
mental implications that are important for the understanding of
mechanisms regulating autoimmune responses. In vivo studies
with the administration of the sICAM-1/Ig protein will be
rewarding.

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