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CTLA-4 Differentially Regulates T Cell Responses to Endogenous Tissue Protein Versus Exogenous Immunogen

Lucy S. K. Walker, Lara J. Ausubel, Anna Chodos, Nyree Bekarian, and Abul K. Abbas

CTLA-4 has long been recognized as a negative regulator of T cell function (1, 2). However, it is not clear whether the primary role of this pathway is to dampen T cell reactivity to self proteins or to modulate the responses of T cells to all Ags, including foreign proteins. Interfering with CTLA-4 function exacerbates autoimmune diseases (3, 4) and CTLA-4 knockout mice exhibit lethal autoimmune pathology (5, 6), but precisely how CTLA-4 normally functions in vivo to curb the pathogenicity of self-reactive T cells has also not been clearly established.

Despite the dramatic phenotype of the knockout, restricting the specificity of CTLA-4−/− cells in TCR-transgenic mice reveals a surprisingly mild alteration in their response to immunization with protein in adjuvant (7). Furthermore, the response of CTLA-4-deficient T cells to viral infection closely mimics that of wild-type T cells in mixed bone marrow chimeras (8). Taken together, these data suggest that the predominant effect of CTLA-4 deficiency or blockade appears to be to unleash T cell responsiveness to "tolerogenic" Ag (e.g., soluble peptide i.v.) (7, 9) or self Ag (5, 6) rather than to modulate responses to immunogenic Ag (e.g., peptide plus adjuvant or microbes). One caveat to the use of i.v. soluble protein to mimic self Ag is that it is unclear whether the former is subject to the same processing and presentation events as a tissue-expressed self protein. To further dissect the types of antigenic encounter that are controlled by CTLA-4, we have developed a model in which a known protein (hen egg lysozyme; HEL) is transgenically expressed as a tissue self Ag and can also be administered exogenously by immunization in adjuvant. By introducing naive HEL-specific T cells from class II-restricted TCR transgenic wild-type or CTLA-4−/− mice, we have been able to assess the relative ability of CTLA-4 to regulate responsiveness to either source of protein. We show that when T cells are exposed to HEL both as a tissue Ag and an administered immunogen, CTLA-4 dramatically limits the local expansion to tissue Ag, but only marginally affects the T cell response to immunizing Ag. In the absence of the CTLA-4 pathway, failure to limit the local accumulation of Ag-specific T cells is associated with the onset of aggressive tissue destruction. We discuss the implications of these results for the pathogenesis of tissue autoimmunity.

Materials and Methods

Mice

3A9 TCR-transgenic mice (wild type or CTLA4−/−) and rat insulin promoter (RIP)-HEL mice (obtained from C. Goodnow, John Curtin School of Medical Research, Canberra, Australia) were maintained on an MRL background. Animals were housed in the University of California (San Francisco, CA) animal facility in accordance with university guidelines and used between 6 and 12 wk of age. Mice were genotyped using PCR and flow cytometry.

T cell transfers

Combined lymph nodes (LN) (axillary, inguinal, brachial, and mesenteric) from 3A9/wild-type or 3A9/CTLA-4−/− mice were stained with the clonal Ab, 1G12, and the number of T cells expressing the transgenic TCR was assessed by flow cytometry. The indicated number of 1G12-positive cells was transferred into recipient mice by tail vein injection. Where indicated, cells were incubated before transfer with 1 μM CFSE (Molecular Probes, Eugene, OR) for 10 min at room temperature followed by two washes with RPMI supplemented as below.

Immunization

HEL protein (Sigma-Aldrich, St. Louis, MO) was prepared emulsified in IFA or CFA (Difco, Detroit, MI) or alum-precipitated or in buffered saline and 100 μg were administered i.p. 24 h following adoptive transfer of 3A9 T cells.

Flow cytometry

At the indicated time point, mice were sacrificed and cells isolated from the relevant lymphoid tissue for analysis. Spleen samples were treated with Tris ammonium chloride to lyse RBCs. Cells were preincubated with anti-CD16/CD32 (mouse FcR) before staining with the clonal Ab to the 3A9 TCR (1G12-biotin; purified from cell culture supernatant), streptavidin-PE, and CD4-PerCP. All Abs were purchased from BD PharMingen (San Diego, CA) unless otherwise indicated. For analysis of CFSE profiles, samples were pooled from two to six mice.
Blood glucose

Blood glucose levels were measured every 3–4 days (Glucometer Elite XL; Bayer, Elkhart, IN) and mice were considered diabetic following two consecutive readings of >250 mg/dl.

Histology

H&E staining of sections from paraffin-embedded tissues was performed according to standard procedures. Pancreatic islets were scored by eye based on infiltration (none, peri-islet, or invasive) with at least 15 islets being scored per individual mouse. Results are expressed as percentage of total islets counted.

In vitro restimulation

Pooled pancreatic LN, or draining (auxiliary and inguinal) LN from recipients of 3A9/wild-type or 3A9/CTLA-4<sup>−/−</sup> cells that were immunized s.c. with 100 μg alum-precipitated HEL were stained with 1G12 and CD4 Abs and purified by high speed cell sorting (MoFlow; Cytomation, Fort Collins, CO) (5–15 mice/group). 1G12<sup>+</sup> CD4<sup>+</sup> cells (4 × 10<sup>6</sup>) were then cultured with 1.2 × 10<sup>6</sup> spleen cells from nontransgenic mice in 0.2 ml of RPMI 1640 supplemented with 1 mM L-glutamine, penicillin, streptomycin, non-essential amino acids, sodium pyruvate, HEPES (all from Life Technologies, Grand Island, NY), 5 × 10<sup>−8</sup> M 2-ME, and 10% FBS (Sigma-Aldrich) containing the indicated concentration of HEL<sub>61</sub>–<sub>62</sub> peptide. Proliferation assays were pulsed with 1 μCi [<sup>3</sup>H]thymidine (NEN, Boston, MA) for the final 7–8 h of the 72-h period, and incorporated radioactivity was measured in a Betaplate scintillation counter (Amersham Pharmacia Biotech, Piscataway, NJ). Supernatants were removed at day 3 for the assessment of cytokine production by ELISA.

Results

T cell cycling to endogenous vs exogenous Ag

We hypothesized that the way in which Ag is processed and presented might influence the ability of CTLA-4 to regulate T cell responses to the Ag. Therefore, we set out to compare the role of CTLA-4 in controlling T cell proliferation to HEL protein expressed as a tissue Ag vs HEL protein administered exogenously in adjuvant. To analyze T cell proliferation to endogenous tissue protein, mice expressing HEL in pancreatic β cells under the control of the RIP (RIP-HEL mice) were adoptively transferred with CFSE-labeled HEL-specific (3A9) CD4 T cells that were either wild type or CTLA-4<sup>−/−</sup>. Both 3A9/wild-type and 3A9/CTLA-4<sup>−/−</sup> cells were phenotypically naive before transfer as evidenced by high CD62L expression (data not shown). On day 10 after adoptive transfer, mice were sacrificed and the CFSE profiles of the 3A9 T cells isolated from the pancreatic LN or control (inguinal) LN were assessed. A proportion of the 3A9 cells isolated from the pancreatic LN had divided as evidenced by a loss of CFSE staining, while those isolated from the inguinal LN remained undivided (Fig. 1A). These data support the notion that pancreas-derived self proteins are brought to the draining LN, presumably by APCs providing surveillance in the pancreas, and presented to naive T cells (10, 11). Strikingly, there was a clear increase in cell division in the pancreatic LN if the transferred 3A9 cells were CTLA-4-deficient. This suggests that CTLA-4 engagement negatively regulates T cell proliferation in response to tissue-derived Ag. To assess the role of CTLA-4 in controlling T cell proliferation to exogenous Ag, 3A9/wild-type or 3A9/CTLA-4<sup>−/−</sup> cells were transferred to nontransgenic recipients that were either left untreated or immunized i.p. 24 h later with alum-precipitated HEL protein. By day 10 after adoptive transfer, all the 3A9 T cells had become CFSE negative (data not shown). Therefore, we examined the response at day 3 when individual peaks of CFSE could still be discerned. Although there was a slight increase in cell division if the transferred cells lacked CTLA-4, the difference in CFSE profiles between 3A9/wild-type and 3A9/CTLA-4<sup>−/−</sup> cells was not as marked as in the response to tissue Ag. Importantly, even extensive titration of the dose of HEL protein used for immunization failed to reveal a larger difference between the response of 3A9/wild-type and 3A9/CTLA-4<sup>−/−</sup> cells (data not shown). This suggests that the regulation of T cell cycling by CTLA-4 is more

![FIGURE 1](http://www.jimmunol.org/)

Proliferation of 3A9 T cells in response to endogenous Ag vs exogenous Ag. A, A total of 2 × 10<sup>6</sup> CFSE-labeled 3A9/wild-type or 3A9/CTLA-4<sup>−/−</sup> T cells were adoptively transferred into RIP-HEL mice. On day 10, pancreatic and inguinal LN were harvested and stained for CD4 and the 3A9 TCR. The CFSE profile of gated CD4<sup>+</sup> 3A9<sup>+</sup> T cells is shown. B, A total of 2 × 10<sup>6</sup> CFSE-labeled 3A9/wild-type or 3A9/CTLA-4<sup>−/−</sup> T cells were adoptively transferred into nontransgenic recipients that were immunized where indicated 24 h later with 100 μg alum-precipitated HEL protein i.p. On day 3, spleens were harvested and the cells stained as described above. Plots show the CFSE profiles of gated CD4<sup>+</sup> 3A9<sup>+</sup> T cells. Two separate experiments are shown that are representative of four.
profound during T cell responses to tissue Ag than during T cell responses to immunizing Ag.

**CTLA-4 regulation of cycling limits T cell accumulation in response to tissue Ag**

We next analyzed the ability of CTLA-4 to control the responses of T cells exposed to both an endogenous and exogenous source of Ag in the same mouse. 3A9/wild-type or 3A9/CTLA-4−/− T cells were transferred to RIP-HEL mice that were immunized 24 h later with alum-precipitated HEL protein. Similar to the data presented in Fig. 1, the clearest difference between the CFSE profiles of 3A9/wild-type cells compared with 3A9/CTLA-4−/− cells was in the pancreatic LN, where the cell cycle progression of the CTLA-4−/− cells was markedly augmented compared with the wild-type cells (data not shown). Consistent with this difference in cell cycling, 3A9/CTLA-4−/− T cells accumulated in the pancreatic LN to a much greater extent than 3A9/wild-type cells, both in terms of percentage (Fig. 2, A and B) and absolute number (Fig. 2C). In the absence of immunization, although the cells divided in response to pancreatic Ag, they did not accumulate in the pancreatic LN, suggesting that they either die in situ or migrate to distal sites (Fig. 2, triangles). The percentage (Fig. 2B) and absolute number (Fig. 2C) of 3A9 T cells in the spleen was increased in i.p. immunized mice compared with immunized mice, consistent with the spleen being a target site for immunization by this route. However, the difference between accumulation of 3A9/wild-type cells and 3A9/CTLA-4−/− cells in the spleen was marginal. Thus, CTLA-4-mediated regulation of cell cycling and T cell accumulation is most marked in the LN draining the site of tissue Ag and appears minimal in the site responding to administered immunogenic Ag.

**Kinetics of T cell accumulation to tissue Ag vs exogenous Ag**

To explore further the differential role of CTLA-4 in controlling responses to Ags processed as self vs those administered as foreign, we performed a kinetic analysis of the T cell response. Again the adoptively transferred 3A9 T cells were provided with both an endogenous (pancreas-expressed) and exogenous (immunizing) source of HEL protein, and the T cell accumulation at the responding sites was assessed. To exclude the possibility that the minimal difference between wild-type and CTLA-4−/− cells observed in response to i.p. immunization was a specific feature of splenic responses, we performed this set of experiments using s.c. immunization and assessed T cell numbers in the draining LN. Although the number of 3A9 T cells in the draining nodes increased at both days 4 and 7 in response to immunization, the difference in expansion between wild-type and CTLA-4−/− cells was small (Fig. 3, middle panels). In comparison there was a clear difference in the number of 3A9/wild-type cells and the number of 3A9/CTLA-4−/− T cells that accumulated at both time points in the pancreatic LN (Fig. 3, left panels). The observation that CTLA-4−/− cells did not show increased expansion in response to the s.c. immunization could not be attributed to preferential migration of primed cells toward the second source of Ag in the pancreas, because equivalent data were obtained after adoptive transfer to nontransgenic recipients (Fig. 3, right panels). Taken together these data indicate a minimal role for CTLA-4 in controlling T cell expansion in response to foreign Ag administered as an immunogen, but a more profound role for regulating accumulation of T cells at the site of tissue Ag expression.

**T cell accumulation to tissue Ag is linked to induction of autoimmunity**

We were next interested to assess whether the accumulation of T cells in the absence of CTLA-4 would have functional consequences for the maintenance of tolerance to tissue Ags. Because the tissue expressing HEL protein in this system is the pancreas, we were able to assess loss of tolerance to the HEL-expressing pancreatic β cells by monitoring the ability of the mice to regulate

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**FIGURE 2.** Increased accumulation of 3A9 CTLA-4−/− T cells in the pancreatic LN. A total of 2.5 × 10⁶ 3A9/wild-type or 3A9/CTLA-4−/− cells were transferred to RIP-HEL mice that were immunized with 100 µg HEL/alum i.p. 24 h later unless otherwise indicated. Representative FACS profiles of pancreatic LN cells (A) and percentage (B) and absolute number (C) of 3A9 T cells in the pancreatic LN and spleen on day 6 are shown. Error bars show SD. Data are from one representative experiment of four.
Nature of immunization influences subsequent response to tissue Ag

In our experimental model, 3A9 T cells failed to accumulate in the pancreatic LN of RIP-HEL mice in the absence of immunization. When a peripheral activation stimulus was provided by immunization, 3A9/CTLA-4−/− cells accumulated in the pancreatic LN and the mice became diabetic, while 3A9/wild-type cells failed to accumulate in the pancreatic LN and did not induce diabetes. These data suggested that the interaction of the T cells with Ag at a peripheral site could affect the subsequent response to encounter with pancreas-derived HEL in the pancreatic LN. We were interested to address whether the nature of the encounter with exogenously provided Ag in the periphery influenced the subsequent response to tissue Ag. Therefore, we altered the immunization conditions by preparing the HEL protein in different adjuvants and examined the effect on accumulation of Ag-specific T cells in the pancreatic LN of mice that had received 3A9/wild-type or 3A9/CTLA-4−/− T cells and had been immunized (HEL/alum s.c.), and analyzed their responsiveness in vitro. We were particularly interested in the ability of the T cells to produce IFN-γ because most studies have indicated a key role for this cytokine (12–14) and its receptor (15–17) in the pathogenesis of diabetes. Despite the striking difference in the ability of wild-type and CTLA-4−/− 3A9 T cells to induce diabetes in RIP-HEL mice, when isolated from the pancreatic LN and analyzed on a per cell basis, both cell types showed comparable proliferative responses to HEL peptide and comparable levels of IFN-γ production (Fig. 5). IL-4 production was below levels of detection in all cases (data not shown). The response of sorted 3A9 T cells isolated from the LN draining the site of immunization confirmed that both wild-type and CTLA-4−/− cells had responded to the immunization, as evidenced by enhanced proliferation and cytokine production compared with unimmunized mice. However, there was no obvious difference in in vitro responsiveness between wild-type and CTLA-4−/− cells. The major effect of the CTLA-4 pathway in T cells responding to tissue Ag thus appears to be to limit T cell expansion and accumulation rather than to alter T cell effector differentiation.
pancreatic LN. As described earlier (Figs. 2 and 3), after immunization with alum-precipitated HEL, there was increased accumulation of CTLA-4<sup>−</sup>/H11002<sup>−</sup>/H11002<sup>−</sup> T cells compared with wild-type cells in the pancreatic LN of RIP-HEL mice, implying that CTLA-4 expressed by wild-type cells was negatively regulating T cell numbers. This was also the case if HEL was administered without adjuvant in buffered saline (Fig. 6A). However, if HEL was prepared in IFA or CFA, no difference was seen between the number of 3A9/CTLA-4<sup>−</sup>/H11002<sup>−</sup>/H11002<sup>−</sup> cells and 3A9/wild-type cells in the pancreatic LN. The overall expansion in the LN draining the site of immunization was equivalent between mice primed with HEL/alum compared with HEL/IFA or HEL/CFA at this time point (data not shown), suggesting that the differences observed in the pancreatic LN could not be attributed to the production of an increased number of effector cells in response to immunization in Freund’s adjuvant. Instead, the data implied that T cells primed by interaction with HEL administered in Freund’s adjuvant were less susceptible to regulation through CTLA-4. Accumulation of 3A9 T cells in the pancreatic LN correlated with induction of diabetes (Fig. 6B). Accordingly, after immunization with HEL/PBS or HEL/alum, only CTLA-4<sup>−</sup>/H11002<sup>−</sup> cells accumulated in the pancreatic LN and caused diabetes, whereas after immunization with HEL/IFA or HEL/CFA both 3A9/wild-type and 3A9/CTLA-4<sup>−</sup>/H11002<sup>−</sup>/H11002<sup>−</sup> cells accumulated (Fig. 6A), and both went on to induce diabetes as evidenced by increased blood glucose levels (Fig. 6B). These data suggest that the context of T cell priming in the periphery may influence the ability of CTLA-4 to regulate subsequent responses to tissue Ags.

**Discussion**

In this study, we have investigated the ability of CTLA-4 to control T cell responses to tissue-expressed Ag and immunized Ag in the same animal. The results suggest that the primary function of CTLA-4 is to control the accumulation of activated T cells at the site of tissue Ag, rather than to alter local responsiveness to Ag administered in immunogenic form. The observation that CTLA-4 is more effective at regulating some responses than others is consistent with previous studies using TCR transgenic mice: T cells lacking CTLA-4 showed only a 2-fold increase in their proliferative response compared with wild-type cells after encounter with immunogenic protein (Ag in adjuvant), but after exposure to “tolerogenic” protein (soluble Ag i.v.), proliferation of CTLA-4<sup>−</sup>/H11002<sup>−</sup>/H11002<sup>−</sup> cells was up to 10-fold greater than that of wild-type cells (7). These data imply that the context in which T cells encounter Ag
can influence the degree of regulation through CTLA-4. The simplest explanation for this is that the APCs presenting immunogenic Ag are phenotypically distinct from those bearing self or tolerogenic Ag. Immunogenic Ag is likely presented by APCs bearing high levels of costimulatory ligands that may override CTLA-4-mediated inhibition, whereas local proliferation in response to self-protein, at least for CD8 cells, has been shown to be CD28-independent (18). This idea is consistent with our observation that the effects of CTLA-4 pathway, in terms of local T cell expansion and diabetes induction (see Fig. 6), are diminished in the presence of strong adjuvants. Thus, while CTLA-4 can clearly function to some extent in the presence of CFA (19, 20), our data suggest that regulation by CTLA-4 is maximal in the absence of adjuvant, for example, during the T cell response to tissue protein.

Our experiments suggest that the chief effect of CTLA-4 in regulating T cell responses to tissue Ag is to control T cell numbers rather than to regulate T cell effector differentiation. CTLA-4 has been shown to regulate expression of the cell cycle proteins cyclin D3, cyclin-dependent kinase 4, and cyclin-dependent kinase 6 (21) and its role in controlling cell cycle progression is now well supported (22, 23). Mathematical studies of T cell proliferation have shown that costimulatory signals causing relatively small effects on the kinetic parameters regulating cell division can result in a large net increase in cell number (24). Thus, a relatively small effect of CTLA-4 at each division could over time result in a large increase in T cell numbers. The cumulative effects of CTLA-4 ligation would be reinforced by the increased levels of CTLA-4 protein expressed with each round of cell cycling (25). The consequences of CTLA-4 engagement are ultimately functionally significant since the presence or absence of this molecule in the adaptively transferred T cells can determine diabetes induction. Thus, the critical function of CTLA-4 may be to restrict the frequency of T cells after activation such that they fail to reach the threshold number required to cause pathogenicity.

In our study, the quantitative effect of CTLA-4 in controlling T cell numbers in the pancreatic LN translates into a qualitative effect on the aggressiveness of pancreatic islet infiltration, and ultimately leads to the dysregulation of glucose homeostasis. The observation that quantitative differences can trigger qualitative effects is reminiscent of observations made when bone marrow chimeras were used to dilute the number of TCR transgenic CD4 T cells specific for a pancreatic islet Ag (26): a high T cell precursor frequency triggered invasive islet infiltration, while at a low precursor frequency only peri-islet infiltration was seen. It also parallels recent studies on CD8 T cells specific for pancreatic-expressed Ag that demonstrate that quantitative differences in CTL precursors can affect qualitative parameters of a response, for example, the dependence on CD4 help (27). The conversion of the relatively innocuous peri-islet infiltration into aggressive infiltration by eliminating CTLA-4 function is consistent with previously published work using another TCR transgenic system (28).

The adoptive transfer of naive HEL-specific CD4 T cells into RIP-HEL recipients can be used to model the thymic release of self-reactive T cells that have escaped central deletion. The fact that such transfers do not induce diabetes suggests that the mere presence of self-reactive T cells in the peripheral repertoire is not in itself sufficient to trigger autoimmune pathology. Such a finding is consistent with the demonstration that T cells specific for self proteins exist in healthy individuals without detrimental consequences (29, 30). In fact, many animal models demonstrate a surprising absence of autoimmune pathology despite a high frequency

FIGURE 5. Responses of 3A9/wild-type or 3A9/CTLA-4−/− T cells recovered from the pancreatic or draining LN. A total of 2.5 × 10^6 3A9/wild-type or 3A9/CTLA-4−/− cells were transferred into RIP-HEL mice that were immunized 24 h later with 100 μg alum-precipitated HEL protein s.c. On day 6, cells were isolated from pancreatic LN or draining (axillary and inguinal) LN, stained for CD4 and the 3A9 TCR and purified by high-speed cell sorting. Day 3 proliferation and cytokine responses of ex vivo isolated 3A9 cells are shown. Equivalent data were obtained in three previous experiments using unsorted cells and correcting for the input number of 3A9 T cells.

FIGURE 6. Influence of immunization conditions on 3A9 T cell accumulation in the pancreatic LN and diabetes induction. A total of 2–2.5 × 10^6 3A9/wild-type or 3A9/CTLA-4−/− T cells were adoptively transferred into RIP-HEL recipients. Twenty-four hours later, mice were immunized with HEL protein in PBS or prepared in the adjuvants indicated. The number of 3A9 T cells in the pancreatic LN on day 6 (A) and blood glucose levels on day 9 (B) are shown. Data are combined from five separate experiments.
of T cells specific for a self Ag (31, 32). This suggests that in the absence of an initiating trigger, such as infection or immunization, the frequency of self-reactive CD4 T cells is uncoupled from the induction of autoimmunity when tissue injury. In some cases, the lack of pathology may reflect immunological ignorance of the self protein. However, in other models, T cell responsiveness has clearly been modulated by exposure to pancreas-derived self Ag. For example, double transgenic mice bearing MHC class II-restricted hemagglutinin (HA)-specific TCR transgenic T cells in addition to pancreatic HA are resistant to lethal shock following injection of HA peptide, unlike their single transgenic TCR-HA counterparts (32). Despite this indication that the T cells have encountered pancreatic HA, progression to diabetes is rare in these double transgenic mice (32). Similarly, our results with RIP-HEL mice show that the absence of diabetes following transfer of naive T cells is not attributable to a lack of exposure to self Ag, since the transferred T cells undergo local proliferation in the pancreatic LN of RIP-HEL recipients.

The role of CTLA-4 in self tolerance was first highlighted by the autoimmune phenotype of the CTLA-4 knockout mouse. Subsequent studies have shown that blocking CTLA-4 Abs enhances autoimmune disease in several experimental models (3, 4). When comparing data derived from different systems, it is useful to remember that injecting anti-CTLA-4 mAb in vivo could potentially modify the function of CD4+CD25+ regulatory T cells in addition to its effects on disease-inducing T cells. One informative model uses BDC2.5 TCR transgenic mice in which the T cells recognize an unknown pancreatic islet Ag (4, 28). In this system, anti-CTLA-4 Abs or transfer of CTLA-4−/− T cells result in accelerated diabetes. Analysis of the Vβ4+ population of T cells, that contain the transferred BDC2.5 cells, failed to show effects on cell division in the pancreatic LN after injection of a blocking anti-CTLA-4 Ab (28). However, these experiments were done by transferring T cells into lymphocyte-deficient hosts; therefore, homoeostatic proliferation may have masked the responses to the self Ag. By varying the timing of anti-CTLA-4 injection, the authors showed that functional engagement of CTLA-4 occurs before the initiation of insulin (4), consistent with the early regulation of Ag-specific T cells in the pancreatic LN revealed in our study.

It is noteworthy that ablation of CTLA-4 alone in T cells specific for a pancreas-expressed protein fails to trigger diabetes in our model, revealing a requirement for an initiating stimulus to tip the balance between tolerance and pathogenicity. The idea that an infectious cue might trigger autoimmunity receives support from a number of models. Diabetes induction in the RIP-lymphoproliferative choriomeningitis virus-gp P14 class I-restricted TCR-transgenic mice after peptide injection is dependent on an additional trigger such as injection of agonistic anti-CD40 Ab or LPS (33). Similarly, there are many examples of diabetes being associated with viral infection both in mice (34, 35) and humans (36–38). In fact, our data suggest that depending on the nature of the inciting trigger, even a functional CTLA-4 pathway may be insufficient to prevent diabetes (Fig. 6).

In summary, this study implicates the CTLA-4 pathway in preferentially controlling T cell responses to endogenous tissue-expressed Ag rather than to immunized Ag. In combination with an inciting trigger such as immunization, the inability of CTLA-4-deficient T cells to regulate responses to tissue Ag results in a dramatic accumulation of T cells in the LN draining the site of tissue Ag expression, and ultimately leads to tissue invasion and injury. Postulated pathogenic mechanisms in autoimmunity are a susceptible genetic background and infections (39). Susceptibility genes may well turn out to encode proteins that participate in peripheral T cell tolerance, exemplified by CTLA-4 in the model we have used. Infection may incite autoimmunity by molecular mimicry or through bystander activation: in our system, this is paralleled by peripheral immunization. Therefore, our model can be used to dissect the complex interplay of endogenous control mechanisms and exogenous inciting triggers in the development of CD4 T cell-dependent organ-specific autoimmunity.

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