Jide Tian
Angelica P. Olcott
Lorraine R. Hanssen
Dan Zekzer
Blake Middleton
Daniel L. Kaufman

Infectious Th1 and Th2 autoimmunity in diabetes-prone mice

Authors' address
Jide Tian, Angelica P. Olcott, Lorraine R. Hanssen, Dan Zekzer, Blake Middleton, Daniel L. Kaufman,
Department of Molecular and Medical Pharmacology, University of California, Los Angeles, California, USA.

Correspondence to:
Daniel L. Kaufman
Department of Molecular and Medical Pharmacology
University of California, Los Angeles
Los Angeles CA 90095-1735
USA
Fax: +1 310 825 6267
e-mail: dkaufman@mednet.ucla.edu

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Summary: In the non-obese diabetic (NOD) mouse, a Th1-biased autoimmune response arises spontaneously against glutamic acid decarboxylase, concurrent with the onset of insulitis. Subsequently, Th1-type autoreactivity spreads intra- and intermolecularly to other β-cell autoantigens (βCAAs), suggesting that a spontaneous Th1 cascade underlies disease progression. Induction of Th2 immunity to a single βCAA results in the spreading of Th2-type T-cell and humoral responses to other βCAAs in an infectious manner. Thus, both Th1 and Th2 autoimmunity can evolve in amplificatory cascades defined by site-specific, but not antigen-specific, positive feedback circuits. Despite the continued presence of Th1 autoimmunity, the induction of Th2 spreading is associated with active tolerance to βCAAs and reduced disease incidence. With disease progression there is an attenuation of βCAA-inducible Th2 spreading, presumably because of a reduced availability of uncommitted βCAA-reactive precursor T cells. We discuss the implications of these findings for the rational design of antigen-based immunotherapeutics.

Introduction

The non-obese diabetic (NOD) mouse spontaneously develops insulitis and insulin-dependent diabetes mellitus (IDDM), whose pathogenesis resembles the human disease process (1–3). We have been studying the immune mechanisms underlying the disease process in these mice with the goal of using this information to develop immunotherapeutics for the prevention and reversal of IDDM in man.

By the beginning of this decade it had been determined that humoral autoimmune responses arose to a number of islet antigens in prediabetic mice and man (4). It was unclear how these autoreactive humoral responses related to the emerging picture that the immunopathology was $CD4^+$ T-cell mediated. It was also unknown whether cellular autoimmune responses arose against β-cell autoantigens (βCAAs) in a defined sequence during the development of IDDM. If autoimmunity began with a specific violation of self-tolerance, it could provide insight into the etiology of the disease and point to specific immunotherapeutic strategies to circumvent the loss of self-tolerance. Alternatively, autoimmune responses might arise to many β-cell

antigens simultaneously due, for instance, to antigen release from β cells that were genetically programed to apoptose, or that were destroyed as a bystander effect of a T-cell response against a viral infection in the islets.

Proliferative T-cell responses to βCAAs arise in a defined developmental sequence

To evaluate these different hypotheses, we characterized the development of splenic T-cell responses in NOD mice from birth to 28 weeks in age to a panel of β-cell antigens which are targets of IDDM-associated autoantibodies. We found that T-cell responses to these antigens arose in a spontaneous manner and in a sequential order (5, 6). Of the tested autoantigens, splenic proliferative T-cell responses were first detected against glutamic acid decarboxylase (GAD) when the mice were 4 weeks of age, concurrent with the onset of insulitis. Subsequently, T-cell autoimmunity spread intermolecularly to other βCAAs such as heat shock protein (HSP), carboxypeptidase H and insulin (*Fig. 1*). These splenic T-cell responses sequentially peaked and then declined before disease onset at approximately 20 weeks in age.

Splenic T-cell responses to GAD also spread intramolecularly as the disease progressed. Using a set of overlapping peptides that span the GAD molecule, we found that the initial proliferative T-cell response (again at 4 weeks of age) involved two adjacent peptides near the GAD carboxy-terminus (GADp35, GADp34) (*Fig. 2*). During the next few weeks, T-cell autoimmunity spread to several additional GAD determinants (e.g. GADp17, GADp6, GADp15). Subsequently, reactivity to GAD peptides declined, paralleling the loss of T-cell responses to the whole protein. The gradual diversification of the primed autoreactive T-cell repertoire in this naturally occurring autoimmune disease parallels similar findings in experimentally induced autoimmune disease (7, 8). Apparently, lymphokine secretion by the first wave of autoreactive T cells in the target organ induces loss of T-cell tolerance to additional antigens, resulting in a cascade of autoimmune responses (7, 9, 10).

The activation of T cells reactive to additional target tissue determinants is likely to promote β-cell destruction, as a number of $CD4^+$ T-cell clones with different specificities which have been derived from islet lymphocytic infiltrates can induce IDDM (11). This spreading of proinflammatory responses among target tissue antigens also confounds the development of immunotherapies designed to delete/inactivate effector T cells.

The key role that the early autoimmune response against GAD plays in the development of IDDM in the NOD mouse was

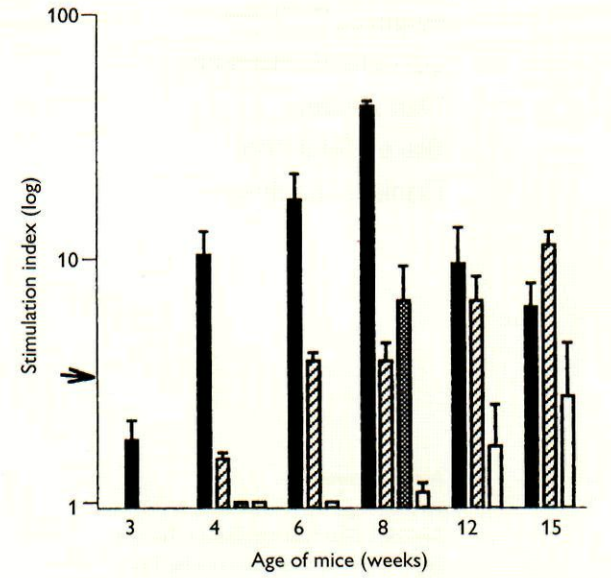

Fig. 1. Proliferative T-cell responses to βCAAs develop spontaneously in NOD mice in a defined chronological order. Antigen-induced blastogenesis was measured in spleen cells from newborn to 28 week old female NOD mice (data from 3–15 weeks are shown). βCAAs included GAD65 (black bars), the immunodominant HSP peptide (51) (striped bars), carboxypeptidase H (gray bars) and insulin (open bars). The data are expressed as stimulation indices (SI) ± standard error. The arrow indicates SI=3, the level of significance. Further details are provided in (5).

demonstrated by our finding that the early inactivation of GAD-reactive T cells circumvented the development of β-cell autoimmunity (5). The GAD-treated mice did not have detectable levels of GAD autoantibodies and their splenic T cells failed to protect recipients in adoptive transfer experiments, consistent with the deletion/inactivation of GAD-reactive T cells (J. Tian, unpublished observations). These data suggested that: 1) the development of autoimmunity to GAD is a key step in the pathogenesis of IDDM in the NOD mouse, and 2) spontaneous autoimmune disease can be prevented by tolerization to a key target antigen. Similar therapeutic results were obtained after the intrathymic administration of GAD to young NOD mice (6).

The many GAD determinants which spontaneously become targets of the autoimmune response (*Fig. 2*) suggests that NOD mice are poorly tolerized to GAD. GAD is not expressed in the thymus and only at low levels in a few peripheral tissues. To evaluate further the role of GAD in the disease process, it would be useful to generate a transgenic NOD

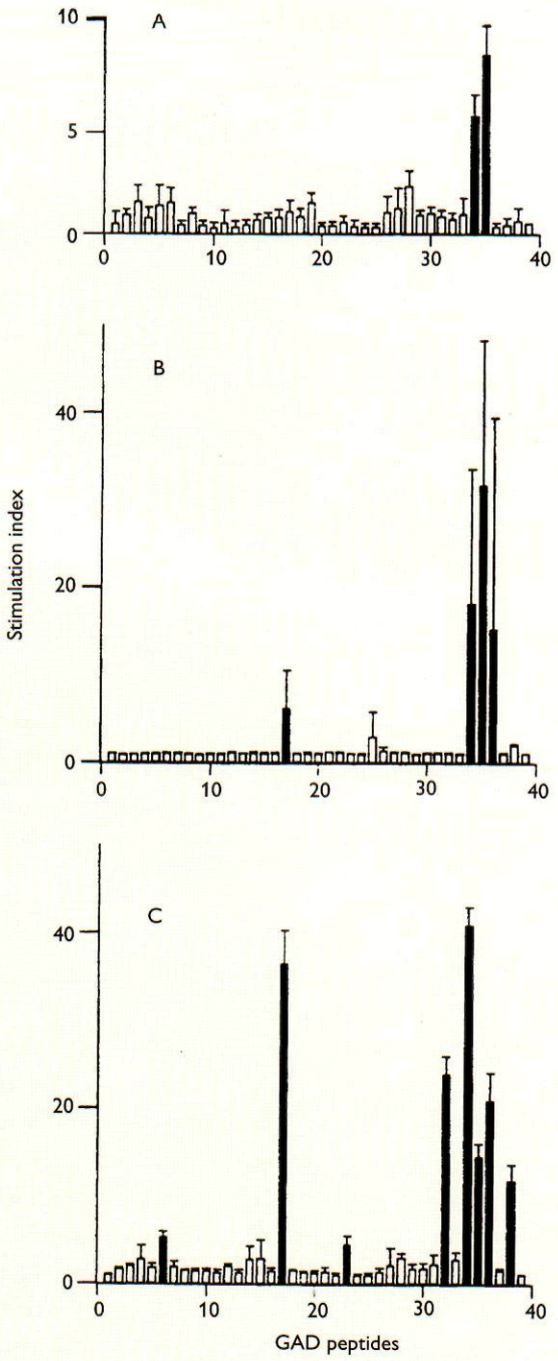

Fig. 2. Intramolecular spreading of autoimmunity within the GAD molecule. Spleen cells from 4–20 week old NOD mice were tested for proliferative responses to an overlapping set of peptides which span the GAD65 molecule, each 20–23 amino acid residues long with overlaps of 5 amino acids. Data shown are for responses from 4 (A), 5 (B) and 7 (C) week old mice. The peptides are numbered successively from the N-terminus. Peptides that triggered stimulation indices >3 are indicated as black bars. The data are represented as the mean SI ± standard error. For further details see (5).

mouse which was tolerant to GAD. However, it may be difficult to achieve tolerance to GAD in NOD mice as these mice have an inherent defect which allows many potentially self-reactive thymocytes to escape from the thymus (12). For example, even though HSP and (pro-)insulin are expressed in the thymus (13) and are ubiquitous in the periphery, tolerance induction to these antigens is incomplete and cellular autoimmunity spontaneously spreads against these antigens. Alternatively, the role of GAD autoimmunity in the NOD mouse could be further addressed through the generation of a β-cell-specific GAD knock-out NOD mouse.

The spreading of Th1 autoimmunity is associated with disease progression

While our initial study showed that a proliferative T-cell response is spontaneously primed to GAD early in the disease process of NOD mice, the nature and dynamics of the autoreactive T-cell pool during disease development remained to be characterized. The antigen specificity and phenotype of T cells involved in the spontaneous autoimmune process has been difficult to address in non-transgenic mice primarily because of the very low frequency of autoreactive T cells within the T-cell pool. Using an ELISPOT assay capable of characterizing T cells at the single cell level (14), we characterized the natural development of β-cell autoimmunity in NOD mice further (15).

When splenic T cells from unmanipulated 4 week old NOD mice (with incipient insulitis) were tested directly *ex vivo*, we detected a unipolar Th1-cell response (with only interferon (IFN)-γ, but no interleukin (IL)-4 or IL-5 spot-forming colonies) to GAD and GADp35 (*Table* 1). Consistent with our previous findings (5), these mice did not yet respond to other GAD peptides (GADp6 and GADp15) or other islet cell antigens (insulin, HSP) which were tested.

By 12 weeks of age, NOD mice exhibited responses to GAD peptides GADp6 and GADp15 in addition to GADp35

Table 1. Spreading of Th2 responses to βCAAs. Unmanipulated NOD mice and mice which had been treated at birth with a βCAA were tested at 4 or 12 weeks in age for splenic antigen-specific IFN-γ, IL-4 and IL-5 T-cell responses using the ELISPOT technique. The data are represented as the mean number of spot-forming colonies per 10^6 splenic T cells. (–) indicates no detectable response. n=6 for each group. The individual variation within each group was less than 15%. Further details are provided in (15).

A. Intramolecular spreading of Th2 responses to GAD determinants

Treatment at birth	Age tested (weeks)	Response to antigens: GADp35			GADp6			GADp15			HEL_{11-25}		
		IL-4	IL-5	IFN-γ	IL-4	IL-5	IFN-γ	IL-4	IL-5	IFN-γ	IL-4	IL-5	IFN-γ
none	4	–	–	86	–	–	–	–	–	–	–	–	–
none	12	–	–	158	–	–	83	–	–	73	–	–	–
HEL_{11-25}	12	–	–	151	–	–	74	–	–	76	68	47	–
GADp11	12	–	–	145	–	–	88	–	–	76	–	–	–
GADp35	12	145	130	63	82	26	56	63	21	55	–	–	–
GADp6	12	103	34	52	101	82	54	30	36	23	–	–	–

B. Intermolecular spreading of Th2 responses to βCAAs

Treatment at birth	Age tested (weeks)	Response to antigens: GAD			HSP			Insulin B chain			β-galactosidase		
		IL-4	IL-5	IFN-γ	IL-4	IL-5	IFN-γ	IL-4	IL-5	IFN-γ	IL-4	IL-5	IFN-γ
none	4	–	–	103	–	–	–	–	–	–	–	–	–
none	12	–	–	365	–	–	130	–	–	70	–	–	–
HEL	12	–	–	350	–	–	105	–	–	68	–	–	–
MBP	12	–	–	359	–	–	116	–	–	64	–	–	–
β-gal.	12	–	–	303	–	–	114	–	–	76	71	43	–
GAD	12	187	113	164	37	26	67	48	48	46	–	–	–
HSP	12	76	34	245	109	66	54	47	17	60	–	–	–
Insulin B	12	65	26	75	25	16	71	137	80	54	–	–	–

(intramolecular spreading) and to insulin B chain and HSP (intermolecular spreading). All of these second-wave reactivities were purely Th1 in nature (*Table 1*). The frequency of GAD-reactive T cells in the spleens of 4–18 week old NOD mice constituted nearly 1 in 10^3 cells, almost two orders of magnitude higher than T cells reactive to control self-antigens or foreign antigens, consistent with clonal expansion (5, 15, 16). Moreover, the GAD reactivity resided within the L-selectin$^-$ fraction of CD4$^+$ cells (5), a phenotype characteristic of primed lymphocytes. Thus, at the single cell level, the spontaneously developing autoimmune process is characterized by the spreading of unipolar Th1-type anti-βCAA reactivity. These data support the notion that a cascade of Th1 autoimmunity against β-cell antigens drives disease progression in IDDM. Whether the splenic T-cell population we have characterized faithfully represents the islet-infiltrating T-cell population remains to be determined.

Spreading of Th2 autoimmunity

We hypothesized that Th2 autoimmunity, like Th1 autoimmunity, might also spread, since the IL-4 produced by Th2 cells is itself a Th2 differentiation factor (17–20). We tested this hypothesis by inducing Th2 immunity to a single βCAA and characterizing the development of T and B-cell responses to unrelated βCAAs.

NOD mice were neonatally treated with control antigens or βCAAs in incomplete Freund's adjuvant (IFA), a protocol which induces vigorous Th2 responses (14) and their splenic T-cell responses against a panel of βCAAs were characterized by ELISPOT. Mice which received the control antigens murine myelin basic protein (MBP), hen egg white lysozyme (HEL) or β-galactosidase displayed vigorous unipolar Th2 responses to the injected antigen (but no IL-4 or IL-5 responses against βCAAs) throughout the course of the disease process. Notably, the induction of Th2 immunity to these non-target tissue antigens did not affect the spontaneous development of Th1-biased anti-βCAA responses (*Table 1*), nor disease incidence.

When NOD mice which had been treated at birth with a single βCAA peptide (GADp35, which contains the earliest known βCAA target determinant) were tested at 12 weeks of age, they displayed clear IL-4 and IL-5 responses not only to the injected peptide, but also to other GAD peptides (GADp6 and

GADp15) which contain later target determinants, indicating Th2-type intramolecular spreading (*Table 1A*). Similarly, neonatal treatment with GADp6 led to the spreading of Th2 immunity to GADp35 and GADp15, indicating that primed Th2 responses can spread to other autoantigen determinants, independent of the order in which spontaneous autoimmune responses arise to these determinants (*Table 1*) (*Fig. 2*) (5, 15). Furthermore, injection of one of the βCAAs (GAD, HSP or insulin B chain) led to the development of Th2 autoimmunity to other unrelated βCAAs (intermolecular spreading) (*Table 1B*), creating an amplificatory cascade of this anti-inflammatory limb.

Notably, following neonatal treatment with a GAD peptide, autoreactive T-cell responses against the injected peptide, as well as uninjected GAD peptides, developed predominantly toward the Th2 phenotype through the intramolecular spreading of Th2 immunity (*Table 1A*). However, while GAD treatment induced significant intermolecular spreading of Th2 responses to unrelated βCAAs (HSP and insulin), Th2-type autoimmunity to these unrelated βCAAs did not become predominant (*Table 1B*). The ability of Th2 immunity to spread more readily intramolecularly than intermolecularly following GAD or GAD-peptide treatment may be due to the induction of a strong anti-GAD B-cell response which efficiently captures and presents GAD determinants and preferentially promotes Th2 immunity (21).

While Th2 immunity spreads readily among target tissue autoantigens following neonatal antigen treatment, it failed to spread to non-target tissue antigens (MBP, HEL or β-galactosidase). In other strains of normal mice, βCAA treatment induced Th2 immunity only to the injected antigen and failed to spread to other β-cell antigens. Thus, the spreading of Th2 immunity is limited to target tissue antigens and is dependent upon a local inflammatory process.

Antigen treatment accelerates the spreading of autoimmunity

Surprisingly, when we characterized splenic T-cell responses in neonatally βCAA-treated NOD mice at earlier time points, we observed that the antigen treatment had accelerated the spreading of autoimmunity (J. Tian, manuscript submitted). For example, NOD mice which were treated with GAD in IFA at birth displayed clear T-cell responses to HSP and insulin at 4 weeks of age – in control NOD mice, spontaneous T-cell responses to HSP and insulin are not detected until a few weeks later (*Fig. 1*) (*Table 1*). Unlike the Th1-biased responses against βCAAs in control NOD mice, the accelerated responses against HSP and insulin in the GAD-treated mice were highly Th2-biased; however, these mice displayed mixed Th2 (predominately) and Th1 responses against GAD itself. Similarly, mice which were neonatally treated with either HSP or insulin B chain displayed accelerated responses against both of these antigens which were primarily of the Th2 type; however, they displayed a mixture of Th1 (predominantly) and Th2 responses against GAD at 4 weeks of age. Thus, following neonatal antigen treatment with different βCAAs there was an acceleration of the usual pattern of determinant spreading, with the accelerated immune responses against GAD having a large Th1 component – indicating that some islet perturbation drives the early development of Th1 autoimmunity against GAD.

As discussed above, when neonatally βCAA-treated mice reached 12 weeks of age they displayed mixed Th1 and Th2 responses to all of the tested βCAAs. Thus, despite displaying predominantly Th2 responses to HSP and insulin at 4 weeks of age, they developed significant Th1 responses to these antigens shortly thereafter.

Apparently, despite the early induction of a cascade of Th2 responses to βCAAs through neonatal antigen treatment, some inherent dysfunction broadly promotes the spreading of Th1-type autoreactivity to βCAAs. However, the Th1 responses against all tested βCAAs were significantly reduced in βCAA-treated animals relative to control groups (*Table 1*). It is unclear whether the autoreactive Th1 arm was reduced because the cascade of Th2 responses antagonized Th1 development and function or because it depleted the pool of uncommitted βCAA-reactive T-cell precursors that could be primed towards a Th1 phenotype.

Th2 spreading leads to the transmission of active tolerance and is associated with reduced disease incidence

The spreading of Th2 immunity in βCAA-treated neonatal NOD mice was associated with greatly reduced proliferative T-cell responses to the injected antigen as well as to unrelated βCAAs (15). A similar inhibition of proliferative responses was observed following βCAA treatment at 6 weeks of age, well after the onset of insulitis and the establishment of Th1-biased autoimmune responses (16, 22). Since ELISPOT analysis in both studies revealed that substantial Th1 responses against βCAAs were still present, the virtual lack of proliferative responses to βCAAs is likely to reflect Th2 (or some other induced regulatory cell) antagonism of Th1 function.

Moreover, following neonatal treatment with GAD in IFA, NOD mice which were subsequently challenged with GAD or insulin B chain in complete Freund's adjuvant (CFA) displayed

no lymph node T-cell response to GAD (confirming the induction of tolerance), as well as greatly reduced responses to insulin B chain (Fig. 3). Similarly, lymph node T cells from NOD mice which were treated at birth with insulin B chain in IFA were silent to both insulin B chain and GAD in CFA challenge. Thus, the spreading of Th2 autoimmunity following autoantigen treatment can lead to the transmission of active tolerance to other unrelated target tissue antigens.

Our studies, as well as those of others, have shown that a number of different βCAAs can effectively prevent disease onset when administered to young NOD mice. Our findings that early βCAA treatment can induce the spreading of active tolerance and cause Th2-type humoral responses to become predominant (see below), even after Th1-biased responses to βCAAs have developed, suggest that Th2 immunity can become functionally dominant and is a potent regulator of disease outcome. The infectious nature of Th2 immunity and the resulting transmission of active tolerance among target tissue antigens may underlie previous observations of "infectious tolerance" (23, 24), and may explain why different autoantigens can be successfully used for immunotherapy early in the disease process.

Th2 spreading promotes humoral autoimmunity

Analysis of humoral responses showed that whereas unmanipulated and control antigen-treated NOD mice had low levels of GAD and insulin autoantibodies, animals treated neonatally with GAD had elevated autoantibodies to both GAD and insulin, consistent with the intermolecular spreading of Th2 immunity (15). Similarly, neonatal treatment with insulin B chain raised the titer of GAD-specific antibodies, in addition to the insulin-specific ones. The induced antibodies were of the IgG_1 subclass, characteristic of Th2 responses (17).

Thus, the spreading of Th2 immunity can promote humoral autoimmune responses – which could potentially exacerbate the disease process or lead to new pathologies (25). However, autoantibodies are not considered to be pathogenic in IDDM, and high autoantibody levels to GAD are actually associated with reduced risk for IDDM in NOD mice and man (16, 22, 26, 27). Indeed, in our studies of GAD-based immunotherapy, it is the loss of Th2-type autoantibodies against GAD that is associated with progression to overt disease (16, 22). In Th2-mediated diseases, however, Th2-determinant spreading may contribute to the disease process, perhaps accounting for observations in allergic conditions and following some infections that animals gradually become sensitized to an increasing number of antigens (28).

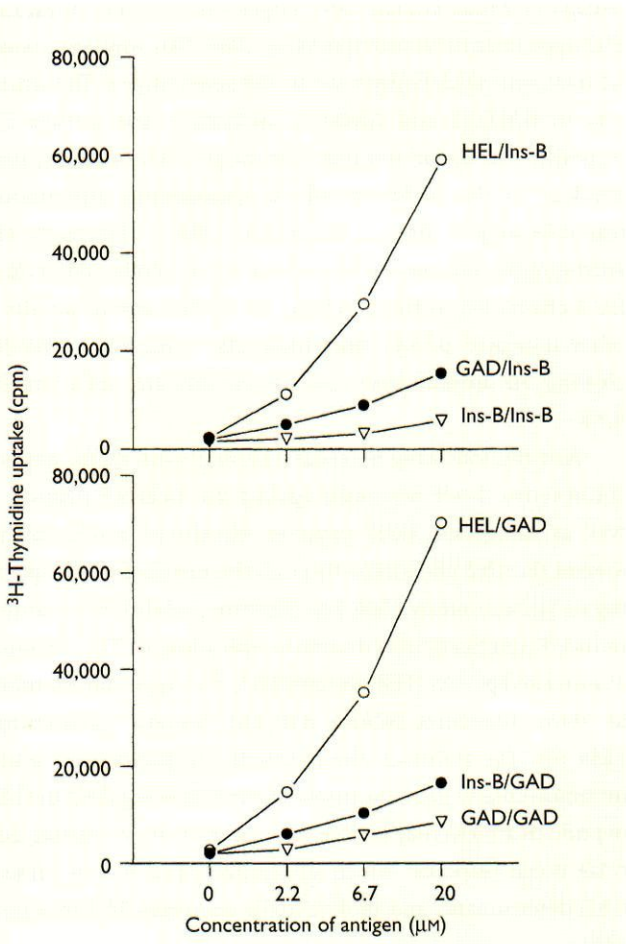

Fig. 3. Transmission of active tolerance. Newborn NOD mice were treated with 100 μg hen egg lysozyme (HEL), GAD, or insulin B chain (Ins-B) in IFA. At 8 weeks of age the mice were immunized with the indicated antigen in CFA and 10 days later their lymph node T cells were tested for proliferative responses to GAD (panel A) or insulin B chain (panel B). Next to each line on the graph, the first antigen indicates the antigen administered at birth; the second antigen is the subsequent challenge. Both neonatal immunization with GAD and insulin B chain had no effect on HEL recall responses. Neonatal administration of HEL antigen did not affect lymph node and splenic T-cell responses to insulin or GAD, which were similar in magnitude to that observed in unmanipulated NOD mice. BALB/c mice treated neonatally with one of the panel of antigens in IFA displayed tolerance to the administered antigen only and not to other βCAAs, consistent with our observation that an induced Th2 responses failed to spread in these mice.

Mechanisms underlying infectious Th1 and Th2 autoimmunity

It is generally accepted that determinants which are efficiently presented induce negative selection of reactive T cells in the thymus or inactivation of reactive T cells in the periphery (29–31). Consequently, only T cells which interact with their

cognate determinant at subthreshold levels for activation are allowed to persist as naive T cells in the periphery. Apparently, these T cells can ignore their cognate determinants in the islets indefinitely. However, once an inflammatory response begins in the islets, naive T cells which were previously interacting with peptide/MHC complexes at subthreshold levels for activation can become activated (7, 32). It is thought that in susceptible animals, the first wave of activated T cells, via the secretion of autocrine cytokines, creates an environment that favors Th1 or Th2-cell differentiation, generating a positive feedback loop of T-cell reactivities, leading to the spreading of Th1 or Th2 autoimmunity among target tissue determinants (7, 15) (also Lehmann et al. this issue). In addition, cytokine-induced local upregulation of MHC and accessory molecule expression can further promote antigen presentation and the expansion of T-cell autoimmunity. Enhanced expression of B7.1 is associated with the development of Th1 immunity, while the upregulation of B7.2 expression promotes Th2 differentiation (33). Furthermore, the activated T cells may selectively promote a particular type of antigen-presenting cell which may preferentially display different antigen determinants (34–36) and thereby drive the spreading of autoimmunity: IFN-γ promotes the activation of macrophage and dendritic cells, which may preferentially present antigen to Th1 cells, while IL-4 promotes the differentiation and survival of B cells, which may preferentially promote Th2 differentiation (37–39). Thus, through the synergism of immune response elements and via positive feedback, Th1 and Th2 immunity can spread in an infectious manner among target tissue antigens.

We propose that during the earliest stages of the disease process, the first T cells which are spontaneously activated and evade apoptosis (due to the low levels of co-stimulation) are those T cells with the highest avidity for βCAA/MHC complexes. Once a proinflammatory Th1 response takes root, it further promotes an inflammatory environment by cytokine production and the recruitment and activation of APC. Consequently, lower avidity βCAA-reactive T cells can interact with antigen/MHC complexes and co-stimulatory factors at above threshold levels for activation, resulting in the sequential spreading of Th1 immunity among βCAA determinants and the development of insulitis.

Of the βCAAs which have been studied in detail, it is notable that GAD is not expressed in the thymus and is expressed only at low levels in restricted peripheral tissues, whereas (pro-)insulin and HSP are expressed in the thymus (13) and are abundant in the periphery. The differential activation and survival of βCAA-reactive T cells based on their avidity may account for why autoimmunity is first detected to specific GAD peptides and then spreads sequentially intra- and intermolecularly to other determinants. Indeed, GADp35 has high affinity for I-A^{NOD} while insulin B $chain_{9\text{-}23}$ is a weak binder (E.-P. Reich, personal communication). This model could explain why the early inactivation of the GAD response can prevent the spreading of autoimmunity to other βCAAs and the development of insulitis (5).

While about a dozen different βCAAs have been described in IDDM, the vast majority of β-cell antigen determinants do not become targets of the spontaneous autoimmune response. Apparently, the vast majority of potentially βCAA-reactive T cells which have escaped tolerance induction fail to interact with peptide/MHC complexes at levels sufficient for activation even in an inflammatory environment.

Attenuation of inducible Th2 immunity with disease progression

Our recent studies have shown that while the early administration of βCAAs to NOD mice broadly diverts the natural development of potentially pathogenic Th1-biased autoimmune responses toward the Th2 phenotype through Th2 spreading, with disease progression there is a steady decline in the ability of βCAA treatment to promote Th2-type cellular and humoral autoimmunity (J. Tian, D. L. Kaufman, manuscript submitted). Late in the disease process, some βCAAs are still able to induce Th2 responses and Th2 spreading (although to a much lesser extent), while other autoantigens were not. This attenuation of inducible Th2 immunity with disease progression may reflect a reduction in the availability of uncommitted autoantigen-reactive precursor T cells. In addition, early, but not late, βCAA treatment can broadly curtail the recruitment of βCAA-specific T cells into the autoreactive Th1 limb, presumably through inducing Th2 bystander suppression of Th1 development and/or guiding the development of uncommitted βCAA-reactive T cells towards the Th2 phenotype.

Rational design of antigen-based immunotherapeutics

Transgenic animal models have shown that neoantigen determinants which are readily available to the immune system induce tolerance efficiently, while neoantigen determinants which are expressed at low levels in peripheral tissues often have little impact on T-cell education and elicit strong immune responses after immunization (40–45). In autoimmune states, the number of potentially reactive precursor T cells should be unique for each βCAA, depending in part on the extent to which central and peripheral tolerance has been induced and

the degree to which these T cells have been recruited into the autoimmune response. Accordingly, the extent to which autoantigen administration can induce regulatory responses should depend on the administered βCAA and the stage of the disease process. Consistent with these notions, our data demonstrate that there are inherent differences in the frequency of spontaneous autoimmune responses to different βCAAs, as well as in the extent to which T-cell responses can be experimentally primed to these antigens (*Table 1*) (J. Tian, D. L. Kaufman, manuscript submitted).

Early treatment with several different βCAAs has been shown to prevent very efficiently the development of IDDM in NOD mice. The efficacy of immunotherapy declines as it is administered later in the disease process (46, 47). We have found that while the early administration of βCAAs to young NOD mice readily spreads Th2 immunity among the largely naive βCAA-reactive T-cell pool, there is an attenuation of inducible Th2 immunity and Th2 spreading with disease progression. Although other cell types have been implicated as mediating the protective effects of antigen-based immunotherapeutics, the ability to induce these cells is likely to follow similar dynamics to those which we have described for Th2 cells.

Prophylactic treatment during the earliest stages of human autoimmune diseases is not yet feasible, making it crucial to develop therapeutics that are effective late in the disease process. Notably, the different βCAAs vary considerably in their ability to protect transplanted syngeneic islets in NOD mice which have an advanced disease process, and the degree of protection correlates with the extent to which the βCAA treatment induced Th2 spreading at this late stage (16) (J. Tian, D. Kaufmann, manuscript submitted). Moreover, transgenic models of autoimmune disease suggest that the proinflammatory/anti-inflammatory autoreactive T-cell balance is important in determining whether β-cell tolerance is established (48, 49). Finally, Sarvetnick and colleagues have recently shown that the protective effects of an IL-4 transgene which is expressed in the β cells is dependent on the availability of a large population of naive T cells (50). Collectively, these findings suggest that: 1) there are inherent differences in the frequency of T cells that are reactive to different βCAAs; 2) the induction of naive T cells towards an anti-inflammatory phenotype over time in the target tissue may be a fundamental mechanism underlying the protective effects of antigen-based immunotherapeutics, and 3) in advanced stages of autoimmune disease, regulatory responses may be best induced with rare target tissue antigens, cryptic βCAA determinants, or altered peptide ligands thereof, against which large uncommitted T-cell pools are still available.

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