Th1 to Th2 Cytokine Shifts in Nonobese Diabetic Mice: Sometimes an Outcome, Rather Than the Cause, of Diabetes Resistance Elicited by Immunostimulation

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Th1 to Th2 Cytokine Shifts in Nonobese Diabetic Mice: Sometimes an Outcome, Rather Than the Cause, of Diabetes Resistance Elicited by Immunostimulation

David V. Serreze, Harold D. Chapman, Cristina M. Post, Ellis A. Johnson, Wilma L. Suarez-Pinzon, and Alex Rabinovitch

Numerous immunostimulatory protocols inhibit the development of T cell-mediated autoimmune insulin-dependent diabetes mellitus (IDDM) in the nonobese diabetic (NOD) mouse model. Many of these protocols, including treatment with the nonspecific immunostimulatory agents CFA or bacillus Calmette-Guérin (BCG) vaccine, have been reported to mediate protection by skewing the pattern of cytokines produced by pancreatic β-cell autoreactive T cells from a Th1 (IFN-γ) to a Th2 (IL-4 and IL-10) profile. However, most of these studies have documented associations between such cytokine shifts and disease protection rather than a cause/effect relationship. To partially address this issue we produced NOD mice genetically deficient in IFN-γ. Elimination of any of these cytokines did not significantly alter the rate of spontaneous IDDM development. Additional experiments using these mice confirmed that CFA- or BCG-elicited diabetes protection is associated with a decreased IFN-γ to IL-4 mRNA ratio within T cell-infiltrated pancreatic islets, but this is a secondary consequence rather than the cause of disease resistance. Unexpectedly, we also found that the ability of BCG and, to a lesser extent, CFA to inhibit IDDM development in standard NOD mice is actually dependent upon the presence of the Th1 cytokine, IFN-γ. Collectively, our studies demonstrate that while Th1 and Th2 cytokine shifts may occur among β-cell autoreactive T cells of NOD mice protected from overt IDDM by various immunomodulatory therapies, it cannot automatically be assumed that this is the cause of their disease resistance. The Journal of Immunology, 2001, 166: 1352–1359.

1 Insulin-dependent diabetes mellitus (IDDM) in the nonobese diabetic (NOD) mouse model results from autoimmune destruction of pancreatic β-cells mediated by both CD4+ and CD8+ T cells (reviewed in Ref. 1). However, it has been widely reported that the pathogenic activity of β-cell autoreactive CD4+ T cells in NOD mice can be inhibited if the predominant pattern of cytokines they produce is shifted from a Th1 (primarily IFN-γ) to a Th2 (primarily IL-4 and IL-10) profile. These conclusions are primarily based on reports that Th1 to Th2 cytokine shifts are often observed among β-cell-infiltrating T cells of NOD mice that are paradoxically protected from overt IDDM by many Ag-specific or nonspecific immunostimulation protocols (reviewed in Refs. 2–4).

2 While the induction of IDDM resistance in NOD mice by many immunostimulatory protocols has been associated with Th1 to Th2 cytokine shifts among β-cell autoreactive T cells, such alterations have not been demonstrated to be the true cause of disease prevention. One frequently overlooked consideration that may complicate interpretation of these associational studies are reports that Th1 are more prone than Th2 cells to activation-induced cell death (AICD) (6, 7). NOD APCs are characterized by a series of genetically controlled T cell activation defects (8–15). Thus, the impaired stimulatory capacity of NOD APC could preferentially inhibit their ability to induce AICD-mediated deletion of autoreactive T cells without fully abrogating their ability to trigger immunological effector functions. Correcting an impaired ability of APC to trigger AICD-mediated deletion of β-cell autoreactive T cells might provide another explanation for how various immunostimulatory agents inhibit IDDM development in NOD mice. Furthermore, if they are indeed characterized by differential sensitivity to AICD, this deleterious process might preferentially spare β-cell autoreactive T cells, producing Th2 rather than Th1 cytokines. Such an “unmasking event” might be misinterpreted as a Th1 to Th2 deviation among β-cell-infiltrating T cells of NOD mice protected from overt IDDM by various immunostimulatory treatments.

3 Several other factors also call into question whether the pathogenicity of β-cell autoreactive CD4+ T cells in NOD mice can be strictly compartmentalized on the basis of currently defined Th1 and Th2 cytokine production profiles. These include reports of Th1 and Th2 clonotypic T cells isolated from NOD mice that, contrary to expectations, had a respective ability to inhibit or promote IDDM development (16–18). Furthermore, it was recently reported that prior skewing in vitro to either a Th1 or a Th2 cytokine production profile did not alter the ability of an NOD-derived β-cell autoreactive CD4+ T cell clone to passively transfer IDDM.
described above could be gained through analyses of NOD stocks

tory role in promoting or blocking IDDM development in NOD

cytokines associated with Th1 and Th2 responses play an obliga-
current study we used such stocks to determine whether various

$\text{IFN-}\gamma$

were considered diagnostic of diabetes onset. Pancreases from mice as-

determined by dividing the total score for each pancreas by the number of islets
examined. Data are presented as the mean insulitis score $\pm$ SEM for the
indicated experimental group.

\textbf{Treatment of mice with nonspecific immunostimulatory agents}

Four-week-old NOD, NOD.IL4null, NOD.IL10null, and NOD.IFN-γnull
female mice were injected in a hind foot pad with 1.0 mg of heat-killed BCG
vacccine (Connaught, Willowdale, Canada) or 50 $\mu$l of CFA (Sigma, St.
Louis, MO). Previous studies have demonstrated that it is the mycobacte-
rial components of CFA or BCG that elicit IDDM-resistant NOD mice, be-
cause no palliative effects are observed following IFA treatment (32, 33).
Controls consisted of females similarly injected with 50 $\mu$l of saline. Four
mice from each group were sacrificed at 8 wk of age for comparison of
intraislet cytokine mRNA levels as described below. All other mice in each
group were monitored for IDDM and insulitis development.

\textbf{Quantitation of intraislet Th1 and Th2 cytokine mRNA levels}

T cell-infiltrated pancreatic islets were isolated as previously described (34) and
subsequently pooled from four NOD, NOD.IL4null, or NOD.IL10null female mice
that had been treated 4 wk earlier with BCG or CFA. Pools of pancreatic
islets were also isolated from two separate groups of four NOD, NOD.IL4null, or
NOD.IL10null female mice treated 4 wk earlier with saline. Comparison of
these separate samples from two independent groups of saline-treated controls
allowed for an assessment of potential intragroup variation. Levels of IFN-γ, IL-4, IL-10, and cyclo-
phin mRNA transcribed within the islets were assessed as previously
described in radiolabeled semiquantitative RT-PCR assays (35). In addition,
TGF-β mRNA levels were analyzed using the primer pair 5'-TTGGTATC
CAGGGCTTCTCC-3' and 5'-TGAATCCTGTCTTTTGAGC-3'. The
intensity of each radiolabeled signal was measured with a Fujix BAS imag-
ning system (Fuji, Tokyo, Japan) and expressed as phosphor-stimulated lu-
minescence units. Each cytokine signal was normalized as a percentage of
the cyclophilin signal for the same sample. All samples compared were
amplified in the same PCR run to avoid interassay variation.

\textbf{AICD rates among NOD CD4+ T cells functionally activated in a Th1 or Th2 cytokine environment}

NOD CD4+ T cells were purified from splenic leukocytes using a strepta-
vidin-conjugated magnetic bead system (Miltenyi Biotec, Auburn, CA) to
deplete T8+ cells, macrophages/granulocytes, and B lymphocytes that
had been prestained with biotinylated Abs directed against lineage-specific
markers. CD8+ T cells and the macrophage/granulocyte populations were,
respectively, depleted with the mAbs 53-6.72 and M1/70, while B lymph-
ocytes were removed with a goat polyclonal antiserum specific for
mouse Ig molecules (Sigma). The purified CD4+ T cells were suspended
at a concentration of 5 x 10^6/ml in the previously described culture
medium (36). These were seeded into tissue culture dishes that had
been precoated as previously described (37) with 3.125 $\mu$g/ml of the
mAb 145-2C11 (PharMingen, San Diego, CA) capable of activating T cells by bind-
ing the CD3 component of the TCR. To drive the anti-CD3-stimulated
CD4+ T cells into a Th1 or Th2 mode, the medium was further supple-
mented with either 50 U/ml rat recombinant IFN-γ (supplied by P. van de
Meurs, Nijmegen, The Netherlands), IFN-γ neutralizing mAb 11B11 (PharMingen),
or 500 U/ml murine rIL-4 (BioSource, Camarillo, CA) combined with 10.0 $\mu$g/ml of the murine
IFN-γ neutralizing mAb XMG1.2 (PharMingen). After incubation at 37°C
for the indicated period of time, the proportion of CD4+ T cells that had
been driven into apoptotically mediated AICD upon anti-CD3 stimulation
under Th1 or Th2 cytokine conditions was assessed by FACS analysis
(FACScan, Becton Dickinson, San Jose, CA). Apoptotic cells were iden-
tified by positive TUNEL staining using a fluorescein-based in situ cell
death detection kit (Roche, Indianapolis, IN). To determine the cytokine
secretion profile of the surviving CD4+ T cells, they were washed free of
exogenous cytokines, resuspended at a concentration of 5 x 10^6/ml in
medium, and subsequently resituated for 24 h in tissue culture dishes
coated with 3.125 $\mu$g/ml of the CD3-specific mAb. Following this sec-
ondary stimulation, the culture supernatants were assessed with commer-
cially available ELISA kits for IFN-γ, IL-4, and IL-10 concentrations
(PharMingen).
Results

Genetic ablation of IFN-γ, IL-4, or IL-10 does not alter IDDM development in NOD mice

If Th1 and Th2 cytokines, respectively, promote or inhibit IDDM development, it might be expected that disease onset would be accelerated in NOD mice made genetically deficient in IL-4 or IL-10. Conversely, IDDM development could conceivably be impaired in NOD mice made genetically deficient in IFN-γ. To address these possibilities, heterozygous carriers of the inactivated cytokine genes that were also fixed to homozygosity for markers of NOD alleles at all previously identified Idd loci were intercrossed at the N7 (IL4null and IFNgnull) or N8 (IL10null) backcross generation. Data represent the rate of IDDM development in female intercross progeny homozygous for the IL4null, IL10null, and IFNgnull alleles compared with those capable of producing these cytokines (pooled null/+ and +/+) segregants.

Inhibition of IDDM in NOD mice by CFA or BCG treatment does not require IL-4 or IL-10 induction

Given that their β-cell autoreactive CD4+ T cells normally fail to produce significant levels of IL-4 or IL-10 (reviewed in Refs. 3 and 4), it is probably not surprising that IDDM development is not enhanced in NOD females made genetically deficient in these Th2 cytokines. In contrast, there have been many reports that IDDM can be inhibited in NOD mice if a normally absent Th2 response is induced among β-cell autoreactive CD4+ T cells (reviewed in Refs. 2–4). Such an up-regulation of Th2 cytokine production by β-cell autoreactive CD4+ T cells has been proposed to be an essential component of IDDM suppression mediated by the nonspecific immunostimulatory agents CFA and BCG (reviewed in Ref. 2). However, as shown in Fig. 2, treatment with either BCG or CFA effectively inhibited IDDM development in NOD.IL4null female mice (0 vs 70% in controls by 20 wk of age). Similarly, the

As reported by another group (20), there was only a slight retardation in IDDM development in NOD.ILN-γnull females (Fig. 1C). Collectively, these results demonstrated that the rate of IDDM development in NOD mice cannot be prevented by solely eliminating their naturally produced levels of the Th1 cytokine IFN-γ or accelerated by eliminating the Th2 cytokines IL-4 and IL-10.

In FIGURE 1. IDDM development is not significantly modulated in NOD mice made genetically deficient in IL-4 (A), IL-10 (B), or IFN-γ (C). Heterozygous carriers of the inactivated cytokine genes that were also fixed to homozygosity for markers of NOD alleles at all previously identified Idd loci were intercrossed at the N7 (IL4null and IFNgnull) or N8 (IL10null) backcross generation. Data represent the rate of IDDM development in female intercross progeny homozygous for the IL4null, IL10null, and IFNgnull alleles compared with those capable of producing these cytokines (pooled null/+ and +/+ segregants).

FIGURE 2. Inhibition of IDDM development in NOD mice by CFA or BCG treatment does not require induction of the cytokines IL-4 and IL-10. NOD.IL4null (A), NOD.IL10null (B), or standard NOD females (C) were injected in a rear foot pad at 4 wk of age with CFA, BCG, or saline and subsequently monitored for IDDM development. *: IDDM incidence significantly less (p < 0.05, by Kaplan Meier life table analysis) than in saline-treated controls.
**FIGURE 3.** Intraislet TGF-β and IFN-γ mRNA levels are respectively increased and decreased in CFA- or BCG-protected NOD, NOD.IL4<sup>null</sup>, and NOD.IL10<sup>null</sup> mice. RNA was isolated from pooled pancreatic islets of four NOD, NOD.IL4<sup>null</sup>, or NOD.IL10<sup>null</sup> female mice that had been treated 4 wk earlier with CFA or BCG. RNA was also extracted from pools of pancreatic islets isolated from two separate groups of four NOD, NOD.IL4<sup>null</sup>, or NOD.IL10<sup>null</sup> female mice treated 4 wk earlier with saline. Samples were assessed for levels of IFN-γ, IL-4, IL-10, TGF-β, and cyclophilin mRNA transcripts in semiquantitative RT-PCR assays. Cytokine mRNA levels are depicted as percentages of cyclophilin mRNA for the same sample.

Intraislet TGF-β and IFN-γ mRNA levels are respectively increased and decreased in CFA- or BCG-protected NOD, NOD.IL4<sup>null</sup>, and NOD.IL10<sup>null</sup> mice

The studies described above demonstrated that the mechanism by which CFA or BCG treatment inhibits IDDM development does not entail an absolute requirement for IL-4 or IL-10 induction. However, it is possible that following CFA or BCG treatment, β-cell autoreactive CD4<sup>+</sup> T cells unable to produce one of these Th2 cytokines instead up-regulate production of the other in a reciprocal compensatory fashion that is then responsible for the inhibition of IDDM development. This possibility was addressed by semiquantitative RT-PCR analyses of cytokine mRNA levels in pancreatic islets from control and CFA- or BCG-treated NOD, NOD.IL4<sup>null</sup>, and NOD.IL10<sup>null</sup> mice. It should be noted that the quantities of RNA that can be isolated from islets of individual mice is not sufficient to conduct RT-PCR analyses. Thus, to control for intragroup variation, RNA was extracted from the pooled islets of four mice in each treatment group. The validity and reproducibility of this approach are illustrated by the fact that closely matched cytokine mRNA levels were found in pools of pancreatic islets isolated from two independent groups of saline-treated NOD, NOD.IL4<sup>null</sup>, and NOD.IL10<sup>null</sup> female control mice (Fig. 3).

As previously reported by others (35, 39), pancreatic islets from standard NOD mice rendered IDDM resistant by CFA treatment were characterized by higher IL-4 mRNA levels than saline-treated controls (Fig. 3). CFA-treated NOD.IL10<sup>null</sup> mice were also characterized by slightly higher intraislet IL-4 mRNA levels than observed in controls. This latter result is unlikely to be of pathogenic significance, because the level of IL-4 mRNA detected in islets of IDDM-susceptible saline-treated NOD mice was much higher than that observed in CFA-protected NOD.IL10<sup>null</sup> mice. In contrast, intraislet IL-4 mRNA levels were not increased in either standard or IL-10-deficient NOD mice protected from IDDM by BCG treatment. Intraislet levels of IL-10 mRNA were actually reduced in both standard and IL-4-deficient NOD mice protected from IDDM by either CFA or BCG treatment. Thus, the inhibition of IDDM by CFA or BCG treatment is not associated with reciprocal compensatory intraislet increases in IL-4 or IL-10 in NOD mice genetically deficient in either of these Th2 cytokines.

CFA- and BCG-induced IDDM resistance in NOD, NOD.IL4<sup>null</sup>, and NOD.IL10<sup>null</sup> mice was associated with decreased intraislet levels of IFN-γ mRNA (Fig. 3). This may result from the fact that mRNA levels for the cytokine TGF-β, which can suppress IFN-γ production (40) were increased in NOD, NOD.IL4<sup>null</sup>, and NOD.IL10<sup>null</sup> mice protected from IDDM by either CFA or BCG treatment. However, arguing against a TGF-β-mediated suppression of IFN-γ production as being the mechanism by which CFA or BCG treatment inhibits IDDM development is the observation that NOD mice genetically deficient in IFN-γ remain disease susceptible (see Fig. 1).

**Insulitis is significantly decreased in CFA protected NOD, NOD.IL4<sup>null</sup>, and NOD.IL10<sup>null</sup> mice**

The data described above indicate that while intraislet Th1 to Th2 cytokine shifts are associated with CFA- and BCG-mediated IDDM protection in NOD mice, such deviations are unlikely to represent an obligatory mechanism by which these agents inhibit disease. Hence, we also examined whether CFA or BCG treatment might actually inhibit IDDM development by inducing the deletion, perhaps through AICD, of a significant fraction of β-cell autoreactive T cells either before or after they have entered the pancreatic islets. If quantitative decreases in numbers of β-cell autoreactive T cells accounted for the induction of IDDM resistance by CFA or BCG treatment, then protected mice should be characterized by lower insulitis levels than saline-treated controls. Hence, insulitis levels were compared among all CFA-, BCG-, and saline-treated mice depicted in Fig. 2 that remained free of overt...
IDDM at 20 wk of age. Only one saline-treated standard NOD female remained free of overt IDDM at 20 wk of age. Thus, we also examined insulitis levels in two other 20-wk-old NOD females from our research colony that had not yet developed overt IDDM. As shown in Table I, the mean insulitis score in these three NOD control females (3.56 ± 0.36) was significantly higher than that observed in the CFA-treated group (1.83 ± 0.62). Similarly, significantly lower levels of insulitis were observed in NOD.IL4null and NOD.IL10null mice protected from IDDM by CFA treatment than in saline-treated controls. Hence, these evaluations of insulitis development indicate that CFA treatment most likely inhibits IDDM development in standard and Th2 cytokine-deficient NOD mice by inducing quantitative decreases in numbers of pathogenic T cells. In contrast, there were no significant differences in mean insulitis scores between saline- and BCG-treated NOD, NOD.IL4null, and NOD.IL10null mice remaining free of overt IDDM at 20 wk of age (data not shown). These collective data indicate that the mechanisms by which the nonspecific immunostimulatory agents CFA and BCG inhibit IDDM development in NOD mice are not completely identical. However, neither of the mechanisms involved is absolutely dependent upon induction of IL-4 or IL-10, or inhibition of IFN-γ.

Inhibition of IDDM by BCG or CFA treatment is IFN-γ dependent

For the reasons described above, we considered it unlikely that CFA or BCG treatment inhibits IDDM development in standard NOD mice as well those genetically deficient in IL-4 or IL-10 by diminishing production of the Th1 cytokine IFN-γ in pancreatic islets. However, to more rigorously test this question, we determined whether CFA or BCG treatment inhibited IDDM development in NOD.IL4null mice. It was reasoned that if either treatment inhibits IDDM in standard or Th2 cytokine-deficient NOD mice by reducing intraislet levels of IFN-γ, then these protocols should also exert highly protective effects in the NOD.IL4null stock. However, BCG treatment failed to inhibit IDDM development in NOD.IL4null mice (Fig. 4). Thus, the mechanism by which BCG treatment inhibits IDDM development in NOD mice actually requires the presence of IFN-γ, which heretofore was thought to contribute exclusively to pathogenic processes. Interestingly, CFA treatment also inhibited IDDM development much less effectively in NOD.IL4null females (Fig. 4) than in standard NOD mice or those genetically deficient in IL-4 or IL-10 (see Fig. 2). This indicated that the mechanism by which CFA treatment blocks IDDM in NOD mice also operates most efficiently under conditions where IFN-γ can be produced. Hence, while there are some differences, there is also overlap in the mechanisms by which CFA and BCG treatment inhibit IDDM development in NOD mice.

Table I. Insulitis is significantly decreased in CFA protected NOD, NOD.IL4null, and NOD.IL10null mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIS ± SEM in Saline-Treated Controls</th>
<th>MIS ± SEM in CFA-Treated Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOD</td>
<td>3.56 ± 0.36 (n = 3) *</td>
<td>1.83 ± 0.62 * (n = 8)</td>
</tr>
<tr>
<td>NOD.IL4null</td>
<td>3.65 ± 0.36 (n = 2) *</td>
<td>1.40 ± 0.34 * (n = 10)</td>
</tr>
<tr>
<td>NOD.IL10null</td>
<td>3.67 ± 0.47 (n = 2) *</td>
<td>1.59 ± 0.68 * (n = 7)</td>
</tr>
</tbody>
</table>

* Females from the indicated strains were treated with saline or CFA at 4 wk of age. Mean insulitis scores (MIS) in mice remaining free of overt IDDM at 20 wk of age were determined as described in Materials and Methods.

** Value based on one saline-treated control remaining free of overt IDDM through 20 wk of age plus two additional age-matched IDDM-free NOD females identified in the research colony.

*** Significantly less (p < 0.05, Student’s t test) than controls.

FIGURE 4. The mechanism by which BCG and, to a lesser extent, CFA treatment inhibits IDDM development in NOD mice requires the presence of IFN-γ. Female NOD.IL4null mice were injected in a rear foot pad at 4 wk of age with CFA, BCG, or saline and subsequently monitored for IDDM development. *: Marginal statistical difference (p = 0.06, by Kaplan-Meier life table analysis) from saline-treated controls.

TCR stimulation induces lower levels of AICD among NOD CD4+ T cells producing Th2 than Th1 cytokines

While unlikely to represent the mechanism of protection, the question remained as to why Th1 to Th2 cytokine shifts are observed during TCR stimulation.
in islets of standard NOD mice that have been made IDDM resistant by CFA treatment. In nonautoimmune strains it has been reported that Th2 are less prone than Th1 cells to AICD (6, 7). This suggested the possibility that CD4\(^+\) T cells producing Th1 cytokines may indeed be important contributors to autoimmune IDDM in NOD mice, but they are more easily deleted by AICD pathways triggered by CFA treatment than those producing Th2 cytokines. Thus, we determined whether AICD rates varied in NOD CD4\(^+\) T cells that underwent TCR-mediated stimulation under conditions designed to promote either a Th1 or a Th2 response. It should be noted that we used anti-IL-4 in conjunction with IFN-\(\gamma\), rather than IL-12, to elicit Th1 responses, because this combination would result in the maximal cosuppression of Th2 activity. At all time points, lower levels of apoptosis were observed among NOD CD4\(^+\) T cells that had undergone anti-CD3 stimulation under Th2 vs Th1 cytokine conditions (Fig. 5).

Surviving CD4\(^+\) T cells underwent secondary anti-CD3 stimulation to determine their cytokine production profiles. As shown in Fig. 6A, these analyses demonstrated that production levels of the Th1 cytokine IFN-\(\gamma\) did not correlate with the ability of NOD CD4\(^+\) T cells to undergo AICD. Interestingly, higher levels of IFN-\(\gamma\) were produced by NOD CD4\(^+\) T cells initially activated under Th2 rather than Th1 conditions. This might be explained by the fact that NOD CD4\(^+\) T cells are predisposed to Th1 responses (reviewed in Refs. 3 and 4) and a report that ongoing Th1 responses can actually be enhanced by IL-4 (41). However, resistance to AICD did correlate with NOD CD4\(^+\) T cells acquiring an enhanced ability to produce the Th2 cytokines IL-4 (Fig. 6B) and/or IL-10 (Fig. 6C). If there are similar disparities in apoptotic sensitivity in vivo, then islet-infiltrating CD4\(^+\) T cells that are producing Th2 cytokines might be preferentially protected from the deletional events that inhibit IDDM development in CFA-treated NOD mice. Such a difference in T cell death rates could result in an unmasking process that has a secondary appearance of being a Th1 to Th2 cytokine shift.

**Discussion**

Many Ag-specific and nonspecific immunostimulatory protocols that inhibit IDDM development in NOD mice have been reported to do so by eliciting a Th1 to Th2 cytokine shift among \(\beta\)-cell autoreactive T cells (reviewed in Refs. 2–4). However, most of these studies have documented associative rather than causative

![FIGURE 6](http://www.jimmunol.org/)

**FIGURE 6.** Resistance of NOD CD4\(^+\) T cells to AICD correlates with an enhanced ability to produce Th2 cytokines. Purified NOD CD4\(^+\) T cells underwent primary anti-CD3-mediated stimulation under the indicated conditions designed to promote a Th1 or a Th2 response. An aliquot of cells was then assessed for AICD rates as shown in Fig. 6. The remaining CD4\(^+\) T cells were restimulated for 24 h with anti-CD3 alone, and culture supernatants were analyzed by ELISA for IFN-\(\gamma\) (A), IL-4 (B), and IL-10 (C) concentrations. Similar results were obtained in two other experiments.
links between such Th1 to Th2 cytokine shifts and the induction of IDDM resistance. In this study, which used two newly developed stocks of NOD mice that are genetically deficient in IL-4 or IL-10, we found that while the induction of IDDM resistance by CFA and BCG treatment is associated with intraislet Th1 to Th2 cytokine shifts, this is a secondary outcome rather than the cause of disease resistance. Hence, while Th1 and Th2 cytokine shifts may be observed among β-cell autoreactive T cells of NOD mice protected from overt IDDM by various immunomodulatory therapies, it cannot automatically be assumed that this is the cause of disease resistance.

Rather than resulting from a Th1 to Th2 cytokine shift, our data indicate that the induction of IDDM resistance by CFA treatment most likely results from quantitative decreases in levels of islet-resistance. Not automatically be assumed that this is the cause of disease from overt IDDM by various immunomodulatory therapies, it can-

generated, the pathogenic effector functions of IL4null suggested that one mechanism by which BCG treatment might

served in CFA and BCG protected NOD, NOD.IL4null mice. These possibilities are supported by a previous report that an NOD-derived CD4+ T cell clone capable of inhibiting IDDM development did so through the release of TGF-β (18). Similarly, both a transgenic and a gene therapy system that increase pancreatic TGF-β levels have been shown to inhibit IDDM development in NOD mice (42, 43). One way that TGF-β has been proposed to inhibit IDDM development in NOD mice is by inducing a change in the type of APC presenting β-cell Ags to autoreactive CD4+ T cells from B lymphocytes to a myeloid population (44).

While not representing the mechanism of action elicited by treatment with the nonspecific immunostimulatory agents CFA and BCG, other protocols that inhibit IDDM development in NOD mice may well do so by eliciting Th1 to Th2 cytokine shifts within β-cell autoreactive T cell populations. For example, one therapy entailing immunization with peptides derived from the candidate β-cell autoantigen glutamic acid decarboxylase inhibits the development of IDDM in standard NOD mice, but not in the same NOD.IL4null stock used in the current study (32). Collectively, these past studies coupled with our current findings indicate that while some immunomodulatory protocols do indeed block IDDM development in NOD mice through an enhancement of Th2 cytokine production by β-cell autoreactive T cells, in some cases such a cytokine shift is a consequence rather than the cause of protection. Hence, great care should be taken in interpreting the pathological significance of any Th1 to Th2 cytokine shifts that may occur among islet-infiltrating T cells of NOD mice protected from the development of overt IDDM by various immunomodulatory protocols. Furthermore, the alternative interpretation that Th1 to Th2 cytokine shifts among β-cell autoreactive T cells of NOD mice made IDDM resistant by immunostimulation can be an outcome rather than the cause of protection may also be applicable to other autoimmune diseases.

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References


