In Vivo Activity and In Vitro Specificity of CD4+ Th1 and Th2 Cells Derived from the Spleens of Diabetic NOD Mice

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Abstract

$CD4^+$ T cell lines were generated from the spleens of diabetic NOD mice against crude membrane preparations derived from a rat insulinoma. Adoptive transfer of these lines into neonatal mice confirms that overt diabetes is induced by γ-IFN–secreting Th1 cells, whereas transfer of IL-4–secreting Th2 cells resulted in a nondestructive peri-islet insulitis. Analysis of the antigens recognized by individual T cell clones from the Th1 line included reactivity against an insulinoma membrane fraction enriched in proteins of ~ 38 kD. Immune responses to the same antigen preparation have been associated with T cell clones derived from human insulin-dependent diabetes mellitus. The specificity of Th2 cells includes reactivity to a fraction enriched in proteins of 30 kD. The data suggest that in insulin-dependent diabetes mellitus the balance between β cell destruction, associated with intra-islet infiltration, and nondestructive (potential protective) peri-islet insulitis may depend on both the antigens recognized, and the prevailing cytokine environment. (*J. Clin. Invest.* 1995. 95:2979–2985.) Key words: diabetes • autoantigen • autoimmunity • mouse • cytokine

Introduction

Insulin-dependent diabetes mellitus (IDDM)[1] in humans results from a spontaneous T cell–dependent autoimmune destruction of insulin-producing β cells in the pancreas. The non-obese diabetic (NOD) mouse serves as a useful experimental model of the human condition (1). The central role played by T cells in this disease has been shown by the success of immunotherapy directed against individual T cell subsets or their functions. These include anti-CD3 antibody (2) and Cyclosporin A (3) for the treatment of human IDDM, in addition to neonatal thymectomy (4), and antibodies to Thy1, CD3, CD4, and CD8, for the treatment of the animal model (5–9).

To achieve a greater understanding of the pathogenetic process $CD4^+$ T cell clones specific for β cell antigens have been isolated (10–13). The studies of Haskins and colleagues clearly demonstrated that such γ-IFN–secreting (Th1) clones were able to cause IDDM after transfer into neonatal NOD mice (10, 11). Such clones have usually been generated from spontaneously diabetic NOD mice using mixed antigen preparations consisting of either whole islet tissue or extracts from insulinoma cell lines. T cell responses have been reported in the NOD mouse against a number of candidate autoantigens including glutamic acid decarboxylase (GAD), carboxypeptidase H, peripherin, heat shock protein (hsp 60), and insulin (14). However, although pathogenic T cell clones specific for hsp 60 and insulin have been isolated (15, 16), the identity of the antigens recognized by the majority of pathogenic T cell clones remains elusive. The existence of clones that do not recognize these candidate antigens, but do induce disease, would suggest that other novel antigens remain to be discovered.

While there is convincing evidence of CD4-dependent β cell destruction in IDDM, data implicating CD4 T cells in protection from IDDM are also emerging. For instance, in our colony, both male and female mice develop a nondestructive peri-islet insulitis at 4–6 wk of age, but at 30 wk only 15% of males are diabetic, compared with 70% of females. Adoptive transfer studies using young male spleen cells to inhibit the cotransfer of diabetes have indicated a direct role for CD4 cells in the protection of male mice (17, 18). Nevertheless, both males and females exhibit an accelerated onset of IDDM when treated with high dose cyclophosphamide, an agent thought to disrupt immunoregulatory circuits (19). The mechanism responsible for this protection is unknown; however, it has been suggested that changes in the prevailing cytokine environment within the islet may play a role in altering disease susceptibility. For example, animals given complete Freunds adjuvant (CFA) immunotherapy are protected from disease when compared with age-matched controls. The cytokine analysis of their respective islet infiltrates would indicate the accumulation of IL-4–secreting (Th2) T cells in the nondestructive insulitis resulting from CFA therapy (20).

To examine the hypothesis that CD4 T cells play a pivotal role in the selection of autoaggression over tolerance, at least in murine IDDM, we have developed T cell lines and clones from diabetic NOD mice which secrete either γ-IFN (Th1) or

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Received for 10 November 1994 publication and in revised form 23 January 1995.

1. *Abbreviations used in this paper:* CMF, crude membrane fraction; ConA sup., ConA-stimulated rat spleen cell culture; GAD, glutamic acid decarboxylase; hsp, heat shock protein; IDDM, insulin-dependent diabetes mellitus; NMS, normal mouse serum; NOD, non-obese diabetic.

J. Clin. Invest.

0021-9738/95/06/2979/07 $2.00
Volume 95, June 1995, 2979–2985

IL-4 (Th2). The in vivo activity of these lines was evaluated by adoptive transfer to neonatal NOD mice, and their antigen specificities were determined by their reactivity with a panel of proteins derived from the membranes of an in vivo passaged rat insulinoma. The data confirm that pathogenesis is a Th1-dependent mechanism. By contrast, β cell–specific Th2 cells, described here for the first time, induce a nondestructive peri-islet insulitis. The antigen specificities recognized by the disease-inducing Th1 line include responses to antigens of 38 kD. Immune responses to a 38-kD protein are associated with IDDM in humans (21).

Methods

Mice. SPF NOD mice were obtained from a breeding nucleus maintained at the CRC (Northwick Park, United Kingdom), originally provided by Dr. E. Leiter (Jackson ImmunoResearch Labs., Inc., Bar Harbor, ME).

Preparation of insulinoma membranes and subcellular components. Rat insulinoma tissue (22) was propagated by subcutaneous implantation in NEDH rats (23). The tissue was homogenized in isotonic sucrose medium (24) centrifuged at 1,700 *g* for 10 min at 4°C to yield a postnuclear supernatant, which was then centrifuged at 45,000 *g* for 30 min to produce a crude membrane fraction (CMF) used in the majority of these studies. SDS-PAGE was performed on CMF on gels polymerized from 12.5% acrylamide, 0.8% *N,N'* bis acrylamide, using the buffer system of Laemmli. Slices (0.5 cm) of gels were electroeluted (LKB Ultraphore, Bromma, Sweden), and the proteins were precipitated and freed of detergent by precipitation with 6 vol of acetone containing 1 M HCl. Pellets (11,000 *g* for 10 min at 4°C) were washed in 90% acetone, dried, and reconstituted by sonication in 50 μl of 10 mM NH_4HCO_3. Protein assays were performed by the Pierce dye-binding procedure. α-TC2 and β-TC3 cell lines were kindly provided by Dr. D. Hanahan (University of California, San Francisco, CA). AtT20 cells were from the American Type Culture Collection (Rockville, MD). All these were grown under the recommended conditions, rinsed four times in PBS, scraped off the tissue culture dishes, and sonicated (10^7 cells) in 2 ml PBS. The sonicate was separated into a membrane and soluble fraction by centrifugation for 10 min at 17,200 *g* at 4°C in a microfuge.

Production of CD4$^+$ T cell lines and clones. Spleen cell suspensions were prepared from spontaneous diabetic NOD mice. Primary bulk cultures containing 2×10^7 total splenocytes were established in 20 ml of a standard growth medium containing RPMI 1640 (Gibco Ltd., Paisley, United Kingdom), supplemented with 2 mM glutamine (Gibco Ltd.), 5 μg/ml gentamicin (Gibco Ltd.), and 0.7% heat-inactivated normal mouse serum (NMS). 10 μg/ml of CMF was added as a source of islet antigen. After 3 d, cells were diluted to 2×10^6/ml, and the growth medium was supplemented with 10% IL-2–containing supernatant, derived from a 24-h ConA-stimulated rat spleen cell culture (ConA sup.). Growth medium was replenished with 10% ConA sup. every 3–4 d. Subsequent restimulations were performed every 14 d, whereby 2×10^6 T cells were cultured with 3×10^7 irradiated (2,500 rad) NOD splenocytes, derived from 6–8-wk-old male mice, in a final volume of 20 ml containing 10 μg/ml CMF, followed by further expansion with 10% ConA sup. from day 3. After three restimulations, the growth medium was supplemented with 10% fetal calf serum in place of 0.7% NMS. To ensure the derivation of both CD4$^+$ Th1 and Th2 cells, selected bulk cultures were established and maintained in the presence of 20 μg/ml anti–IL-4 antibody (11B11; kind gift from Dr. O'Garra, DNAX, Palo Alto, CA) for six restimulations to enable the outgrowth of γ-IFN–secreting Th1 cells. The absence of γ-IFN in the ConA sup. allowed the outgrowth of IL-4–secreting Th2 cells. T cell clones were derived by limiting dilution, using 10 μg/ml of CMF as antigen, and growth medium containing 0.7% NMS. Expanded clones were subsequently cultured using the protocol outlined above. Control CD4$^+$ Th1 and Th2 cell lines and clones were derived from mice actively immunized with either CFA alone, giving rise to anti-PPD reactive T cells, or with ovalbumin (25).

Determination of Th1 and Th2 cytokine profiles by ELISA. To assign individual T cell lines and clones to either Th1 or Th2 categories, culture supernatants were collected 48 h after restimulation of bulk cultures and assayed for either γ-IFN or IL-4 by ELISA. 96-well Maxisorb plates (Gibco Ltd.) were coated with either 50 μl of 10 μg/ml anti–γ-IFN (H22; kind gift Dr. A. Morgan, Celltech, Slough, United Kingdom) or anti–IL-4 (11B11) in 0.1 M $NaHCO_3$, pH 9.6, for 2 h at 37°C. After washing with 0.1% Tween-20 in PBS, and blocking with 50 μl of 1% BSA in PBS for 30 min, 50 μl of test samples was incubated on coated plates for 12 h at 4°C. Bound γ-IFN was detected by reaction with 50 μl of a rabbit polyclonal anti–γ-IFN antiserum (diluted 1:1,000 in PBS for 1 h at 37°C; kind gift of Dr. J. Tite, Wellcome Research Laboratories, Beckenham, United Kingdom), washed, and further incubated with 50 μl of peroxidase-labeled anti–rabbit antiserum (diluted 1:500 in PBS for 1 h at 37°C). Bound IL-4 was detected with 50 μl of 5 μg/ml biotinylated anti–IL-4 (clone BVD6–24G2; PharMingen, San Diego, CA) for 1 h at 37°C, washed as above, and incubated with 50 μl of streptavidin-peroxidase (1:1,000 in PBS for 1 h at 37°C; Amersham International, Amersham, United Kingdom). Both ELISAs were developed using 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (Sigma Chemical Co., Poole, United Kingdom) as chromogen. Optical densities at 492 nm were converted to biological units by comparison with a standard titration of recombinant cytokine. Th1 lines and clones (2×10^6 cells sampled) produced > 400 U/ml of γ-IFN and < 3 U/ml of IL-4. Th2 lines and clones produced > 15 U/ml of IL-4, with < 3 U/ml γ-IFN.

Adoptive transfer of insulitis and diabetes. To evaluate the in vivo activity of CD4$^+$ Th1 and Th2 cell lines, adoptive transfer experiments were performed into neonatal NOD mice (26). T cells were harvested from bulk cultures 7 d after their last restimulation, and 3×10^6 cells were delivered, by intraperitoneal injection, to 5-d-old NOD mice. A second and third injection of 3×10^6 cells were administered on days 8 and 12, respectively. Monitoring for overt diabetes was performed by regular blood glucose determinations using a glucometer (Ames, Basingstoke, United Kingdom). A reading of > 14 mM/liter was considered diabetic. To determine an insulitis score for all mice, pancreata were excised and fixed in formal-saline, and processed for paraffin embedding. 5-μm sections were cut at three levels and stained with hematoxylin and eosin. The score was calculated as follows: 0, an islet free of pathology; 1, peri-islet infiltrate with 25% of the islet affected; 2, insulitis with 50% the islet affected; 3, 75% of the islet showing intra-islet infiltration; and 4, complete islet destruction. The scores were totaled and expressed as a percentage of the total possible score (grade 4 × number of islets scored).

Proliferative responses to insulinoma subfractions. To determine the antigen specificity of individual T cell clones, proliferation assays were performed using either insulinoma subcellular components or proteins subjected to SDS-PAGE separation and electroelution (see above): 2×10^4 T cells were cultured in flat-bottomed 96-well plates (Falcon, B.D., Oxford, United Kingdom) with 5×10^5 irradiated (2,500 rad) NOD splenocytes, in a final volume of 200 μl of growth medium, containing 0.7% NMS and antigen at either 1 or 10 μg/ml total protein. Proliferation was measured by thymidine uptake. After 60 h, cultures were pulsed with 0.5 μCi of [^{3}H]thymidine for a further 12 h, before harvesting and β-scintillation counting.

Results

In vivo activity of diabetes-associated T cell lines. CD4$^+$ T cell lines were generated from the spleens of diabetic NOD mice by coculture with a CMF derived from an in vivo passaged rat insulinoma. To enable the isolation of both γ-IFN– and IL-4–secreting T cells (so called Th1 and Th2), anti–IL-4 antibody was added to the primary culture of one set of experiments to select for Th1 over Th2. After six rounds of restimulation with the rat insulinoma membrane, followed by expansion in IL-2, each cell line was evaluated for the ability to induce overt

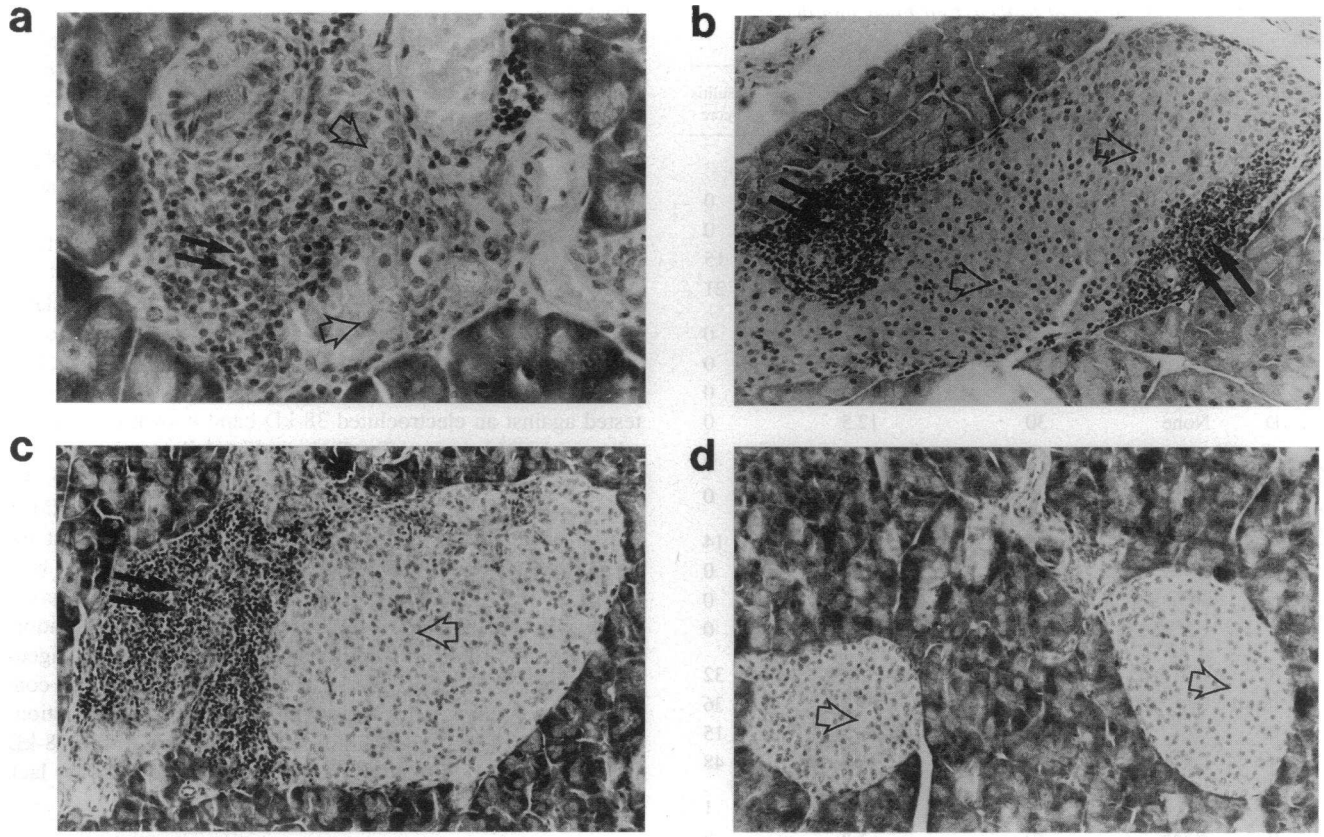

Figure 1. (*a*) NOD diabetic CD4 Th1 line, (*b* and *c*) NOD diabetic CD4 Th2 line, and (*d*) NOD CD4 anti-PPD Th1 line. Neonatal NOD mice received 3 intraperitoneal injections of 3×10^6 NOD T cell lines between days 5 and 12 after birth. Blood glucose levels were monitored regularly to detect overt diabetes. Mice were killed either when acutely diabetic (> 14 mM/liter) or at the end of the experiment, day 30 after transfer. Pancreata were fixed in formal-saline and processed for paraffin sectioning. Insulitis was determined by scoring hematoxylin and eosin–stained sections (see Methods). Open arrows highlight islet cells, black arrows signify mononuclear infiltrate.

diabetes versus peri-islet insulitis by transfer to neonatal NOD mice. Adoptive transfer of 1×10^7 Th1 cells resulted in intra-islet infiltration (Fig. 1 *a*) and overt diabetes within 7–30 d after transfer (Table I, *DIA Th1*). Early onset diabetes was associated with a high mortality, with animals going from normoglycemic to death < 12 h. By contrast, the Th2 cell line only induced mild peri-ductal infiltrates by 15 d after transfer (Fig. 1 *b*). By 30 d, 70% of the islets were associated with a nondestructive peri-islet insulitis (Fig. 1 *c* and Table I, *DIA Th2*). Mice receiving control Th1 and Th2 CD4 cell lines failed to develop significant peri-islet insulitis or overt diabetes by 30 d after transfer (Fig. 1 *d* and Table I). Although the islet antigen reactive T cell lines were maintained in culture using rat insulinoma–derived antigens, their ability to home specifically to NOD islets in vivo implies that the antigens they recognize are present in both mouse and rat β cells.

The antigen specificity of islet reactive T cell clones. To identify the range of islet antigens recognized by both DIA $CD4^+$ Th1 and Th2 T cell lines, these lines were cloned by limiting dilution using the rat CMF as antigen. An initial titration of the CMF used in the bulk cultures, and as a positive control in all assays, suggested that a concentration of 10 μg/ml of CMF represented a submaximal dose of antigen, which would allow for both the detection of antigen enrichment and for positive responses to antigens present at lower concentrations. To determine the tissue distribution of islet-associated antigens, individual T cell clones were tested against 10 μg/ml of either membrane or cytosolic fractions derived from in vitro propagated α-TC2 (murine glucagonoma), AtT20 (murine pituitary cell line), and β-TC3 (murine insulinoma) cells, in addition to secretory granule and cytosolic fractions derived from the in vivo propagated rat insulinoma. Both of the Th1 clones, BDA.1 (TCR Vβ8.2) and BDA.22 (TCR unknown), failed to recognize the control tissues and responded poorly to the in vitro cultured β-TC3 cells (Fig. 2, *a* and *b*). They do, however, react with the granule-enriched fraction derived from the in vivo passaged insulinoma. The poor response against the in vitro propagated insulinoma versus the in vivo grown tumor could reflect differences in their respective phenotypes, in particular, the loss of granulation often associated with in vitro cultured β cells. The Th1 clone BDA.25 (TCR Vβ8.1) and the Th2 clone BDA.7 (TCR Vβ8.2) responded to membranes from β-TC3 cells, in addition to both granule and cytosolic fractions from the rat insulinoma (Fig. 2, *c* and *d*). These data would indicate that Th1 clones BDA.1 and BDA.22 are likely to recognize different antigens to the Th1 clone BDA.25.

Molecular mass determination of T cell reactive islet antigens. To determine the molecular mass of the antigens detected by the T cell clones, rat insulinoma membrane proteins were separated by SDS-PAGE electrophoresis, under reducing conditions, followed by electroelution and acetone precipitation. Fig. 3, *a–c*, shows the molecular mass ranges of antigens (used at

Table I. Adoptive Transfer of NOD T Cell Lines and the Induction of Diabetes

Experiment No.	Cells transferred	Days after transfer	Glucose levels	Insulitis score
			mM/liter	
1. A	PPD Th1*	15	9.0	0
1. B	PPD Th1	15	12.1	0
1. C	DIA Th2‡	15	10.0	15
1. D	DIA Th2	15	10.5	21
2. A	None	30	9.7	0
2. B	None	30	11.6	0
2. C	None	30	8.9	0
2. D	None	30	12.5	0
2. E	OVA Th2*	30	5.1	7
2. F	OVA Th2	30	11.9	0
2. G	PPD Th1	30	8.2	14
2. H	PPD Th1	30	11.1	0
2. I	PPD Th1	30	7.7	0
2. J	PPD Th1	30	7.9	0
2. K	DIA Th2	30	5.0	32
2. L	DIA Th2	30	11.4	36
2. M	DIA Th2	30	8.7	15
2. N	DIA Th2	30	6.6	48
3. A	None	30	6.1	1
3. B	None	30	5.4	6
3. C	None	30	9.7	0
3. D	None	30	7.2	0
3. E	PPD Th2*	30	8.0	7
3. F	PPD Th2	30	6.9	5
3. G	PPD Th2	30	6.4	0
3. H	PPD Th1	30	6.8	0
3. I	PPD Th1	30	5.3	0
3. J	PPD Th1	30	6.5	0
3. K	DIA Th1§	10	Dead	N/A
3. L	DIA Th1	10	20.4	94
3. M	DIA Th1	30	>22 (positive urine from day 25)	88
4. A	DIA Th1	7	Dead	N/A
4. B	DIA Th1	7	Dead	N/A
4. C	DIA Th1	15	> 22.0	100
4. D	DIA Th1	22	14.6	74

* PPD and OVA refer to antigen-specific control T cell clones of either Th1 or Th2 cytokine profiles. ‡ DIA Th2 refers to the Th2 T cell line generated from a diabetic spleen. § DIA Th1 refers to the Th1 T cell line generated from a diabetic spleen. N/A., not applicable.

1 μg/ml) detected by the Th1 clones, BDA.1, BDA.22, and BDA.25, respectively. The antigen detected by BDA.1 is enriched in a fraction with a molecular mass of 34–39 kD. BDA.22 reactivity is contained within the region of 34–57 kD, whereas the BDA.25 antigen is centered on 48–61 kD. By contrast, the Th2 clone BDA.7 failed to react with the electroeluted panel. The antigenic epitope detected by this clone is either denatured under reducing conditions, or has a molecular mass of < 10 kD, as membranes denatured in urea followed by dialysis failed to stimulate BDA.7 (data not shown). However, an alternate Th2 clone, BDA.15, was reactive with a protein of 27–30 kD (Fig. 3 *d*). None of the clones responded to sheep, bovine, or pig insulin, or to three peptide sequences derived from GAD65; the selected peptides have been previously associated with IDDM in the NOD mouse (27).

The antigen specificity of clone BDA.1. The Th1 clone, BDA.1, detected a granule-associated islet antigen of 34–39 kD (Fig. 3 *a*). An equivalent antigen (38 kD) has been associated with T cell responses recovered from diabetic patients to the same rat insulinoma (21, 28). To determine whether clone BDA.1 did indeed react with a 38-kD antigen, this clone was tested against an electroeluted 38-kD band known to stimulate a human IDDM-derived T cell clone. Fig. 4 shows the response of BDA.1 to 10 μg/ml of 33–38 kD and 2 μg/ml 38 kD. Although positive responses were observed against these fractions, the absolute counts were less than that achieved against the unenriched CMF. As the 33–38-kD region was tested at high (10 μg/ml, Fig. 4) and low (1 μg/ml, Fig. 3 *a*) concentrations, it is unlikely that the weaker responses represent supersaturation. Therefore, the results would indicate that some of the antigens purified by SDS-PAGE and electroelution have undergone considerable degradation. Even though BDA.1 reacts with fractions enriched in 38-kD proteins, a direct comparison to the 38-kD antigen detected by human T cells is difficult due to the lack of a highly purified or recombinant source of antigen.

Discussion

The data presented in this study demonstrate that both Th1 and Th2 T cell lines and clones can be isolated from the spleens of diabetic NOD mice. Although both the Th1 and Th2 cell lines have been shown to home to the pancreas causing insulitis after transfer into neonatal NOD mice, only the Th1 T cell line was able to cause IDDM. It is well established that $CD4^+$ T cells act as either the inducers of or direct effectors in β cell destruction in IDDM (29). However, there are also data showing that $CD4^+$ T cells may be able to play a regulatory role in modulating disease onset, as $CD4^+$ T cells from young male NOD mice are able to prevent IDDM. Nondestructive peri-islet infiltrates containing $CD4^+$ T cells are seen in the pancreas of male NOD mice, suggesting that the balance between a regulatory $CD4^+$ T cell population and a pathogenic population may determine disease outcome. Experiments are currently in progress to determine whether the islet reactive Th2 cell line is able to modulate induction of IDDM either spontaneously or via the Th1 cell line.

A role for such Th2 cells in the prevention of IDDM has been indicated by studies of PVG ($RT1^u$) rats rendered diabetic by adult thymectomy and low dose irradiation where disease onset is blocked by infusion of a $CD4^+$ T cell population enriched in Th2 activity ($CD4^+$, $CD45RC^{lo}$) (30). The ability of exogenous IL-4 and IL-10 to prevent the spontaneous onset of IDDM in NOD mice is also consistent with this view (31, 32). Another reason for studying the effect of the Th2 cell on the development of IDDM is the recent report indicating that IL-10 may in some circumstances positively affect disease onset. NOD mice expressing IL-10 under the insulin promoter manifest accelerated insulitis and β cell destruction (33, 34). Local production of IL-10 in this situation therefore may operate as

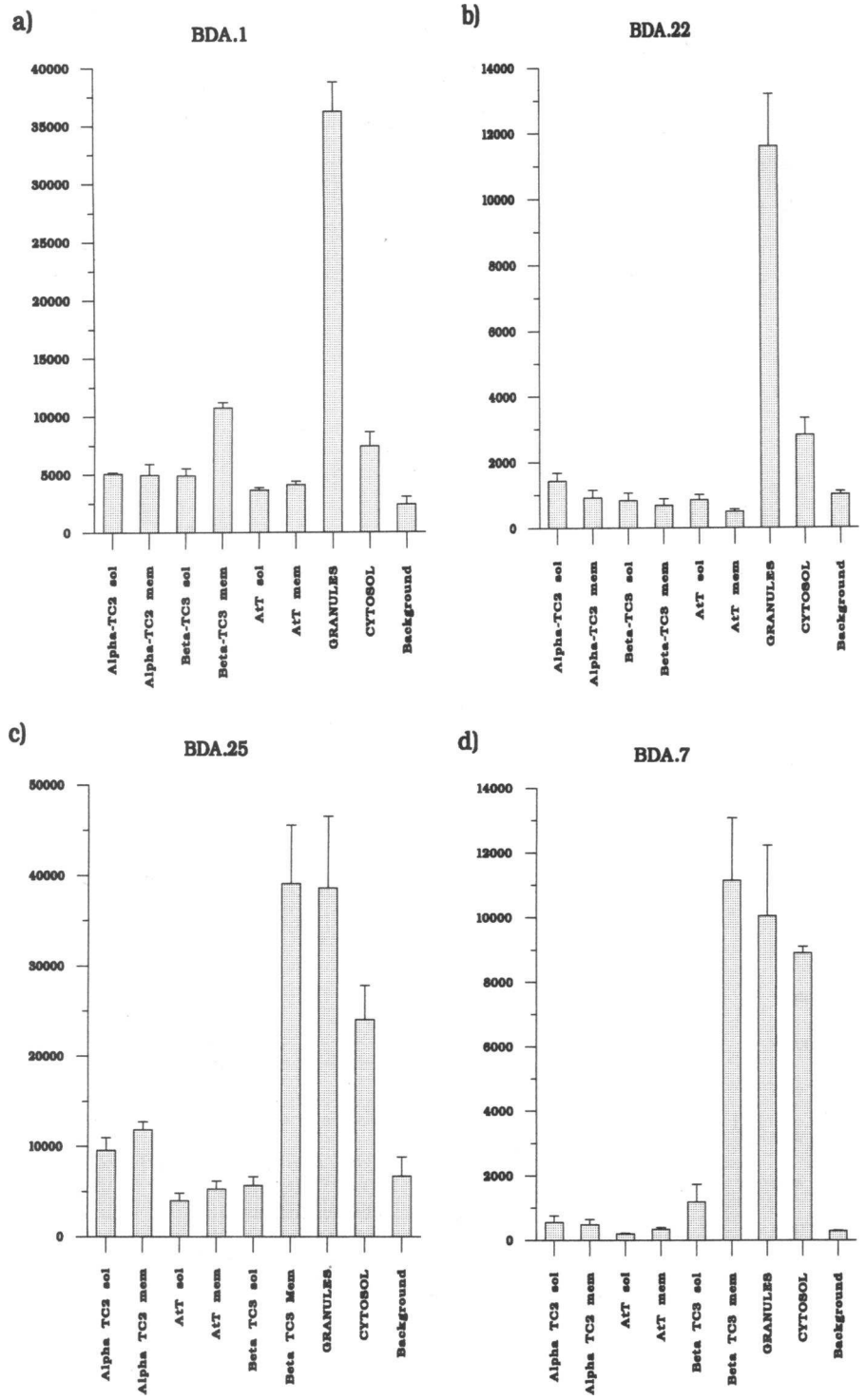

Figure 2. (*a*) BDA.1, (*b*) BDA.22, (*c*) BDA.25, and (*d*) BDA.7. 2×10^4 T cells were cultured with 5×10^5 irradiated NOD spleen cells in the presence of 10 μg/ml of either membranes (*mem*) or cytosol (*sol*) from in vitro cultured α-TC2, β-TC3, or AtT20 cells, in addition to granules and cytosol preparations from the in vivo passaged rat insulinoma. Responses to 10 μg/ml CMF: BDA.1 25,981±721; BDA.22 10,371±940; BDA.25 36,640±6,887; and BDA.7 11,096±1,673.

a chemoattractant, upregulating adhesion molecules on vascular endothelium.

NOD-derived diabetogenic T cells have been described which manifest species- or even strain-specific islet antigen recognition responses (10) while other T cell clones react with mouse or human islet antigens (13). Several of the diabetogenic clones of Haskins and colleagues (10) did not respond to rat islet antigens but did respond to β-TC3, clearly suggesting a different antigen specificity from most of our T cells which have been selected on rat islet antigens. With regard to antigen recognition by the T cell lines and clones described here, although the T cells were maintained on rat insulinoma–derived antigens, the ability of the T cell lines to home to mouse islets in vivo, and in the case of the Th1 cell line, to initiate IDDM, indicates the recognition of epitope(s) conserved between species. T cell clones isolated from both the Th1 and Th2 cell lines were used to carry out a more detailed analysis of islet antigen recognition. Although NOD spleen cells have been shown to

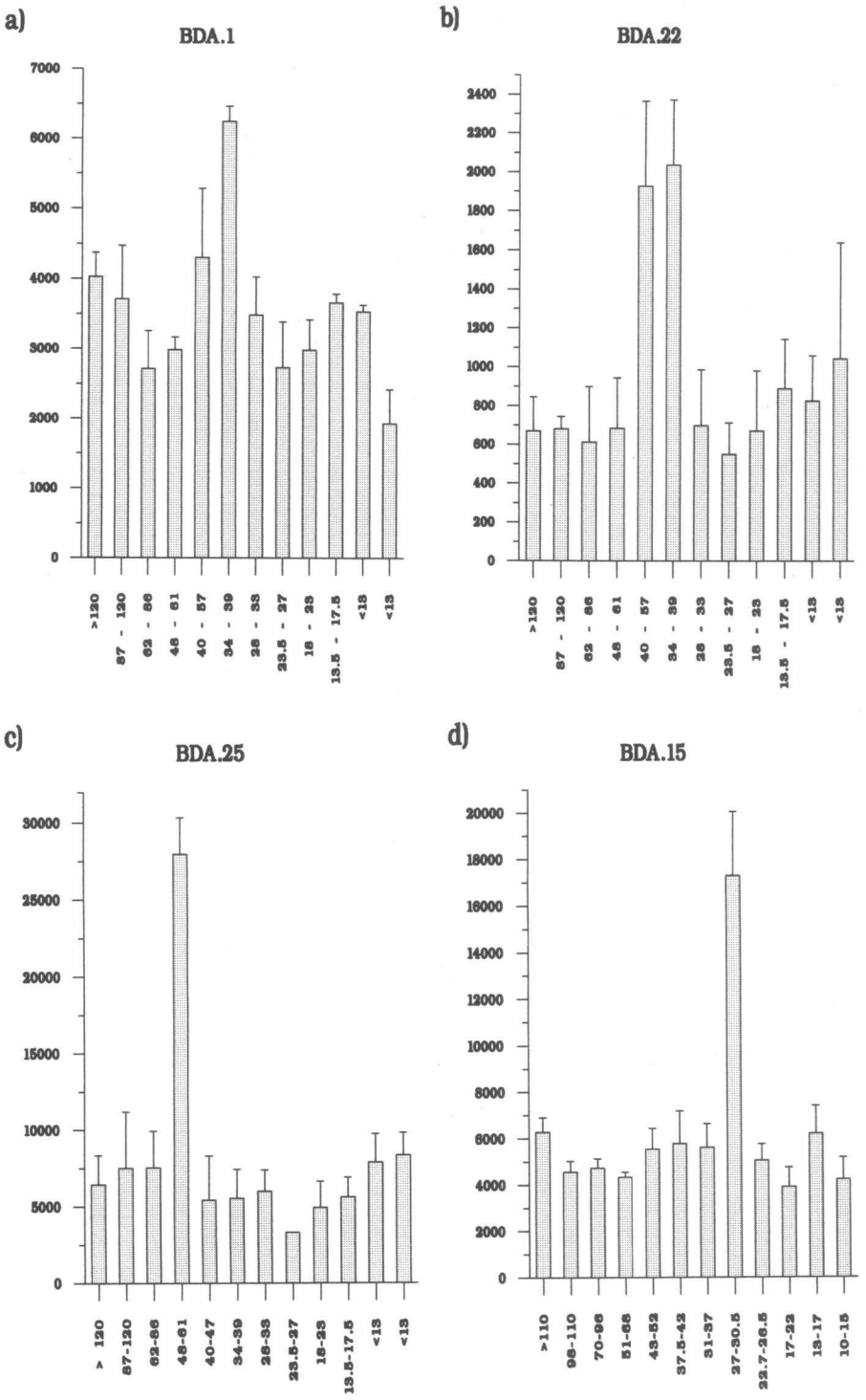

Figure 3. (*a*) BDA.1, (*b*) BDA.22, (*c*) BDA.25, and (*d*) BDA.15. 2×10^4 T cells were cultured with 5×10^5 irradiated NOD spleen cells in the presence of 1 μg/ml of electroeluted proteins, of defined molecular mass (kD), purified from the membranes of the rat insulinoma. Positive control responses are given in legend to Fig. 2, BDA.15 29,463±1,556.

manifest T cell reactivity to some well characterized antigens such as GAD, peripherin, insulin, and hsp 60, those antigens recognized by the majority of pathogenic T cell clones have not been fully characterized. The Th1 cell clones described here recognized islet membrane–associated antigens in the molecular mass ranges of 34–39, 34–57, and 48–61 kD. Gelber and associates (13), who examined the reactivity pattern of NOD T cells to islet antigens, also noted reactivity to proteins of molecular masses 30, 50, and 55–65 kD. Of particular interest is our observation that one of the Th1 clones, BDA.1, recognizes an antigen present in a fraction enriched in the 38-kD antigen known to stimulate a human T cell clone derived from a diabetic patient. However, as the antigen has not yet been fully characterized and is not available as a full-length recombinant protein for comparative study, it is not possible to say whether the same antigen is being recognized by human and mouse T cells. The ability of the individual T cell clones isolated from the diabetogenic T cell line to cause disease is currently under investigation. The recognition of different antigens by the Th2 T cell clones from those recognized by the Th1 clones might enable

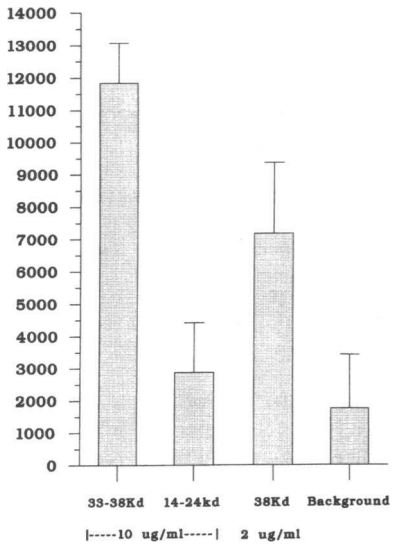

Figure 4. 2×10^4 BDA.1 T cells were cultured with 5×10^5 irradiated NOD spleen cells in the presence of 10 μg/ml of electroeluted proteins of 33–38 kD, 10 μg/ml of 14–24 kD, 2 μg/ml of 38 kD. Background = no antigen. Positive control (10 μg/ml CMF): 38,031±4,640.

the use of these antigens, once identified, to boost a Th2 response in vivo and to perhaps prevent IDDM.

Acknowledgments

The authors would like to acknowledge the support of Dr. E. Simpson, CRC, Northwick Park, London, United Kingdom. We thank Mr. Francis Schindler, Department of Pathology, Royal College of Surgeons, London, United Kingdom, for the preparation and staining of histological material, and Dr. Elaine Bailyes and Ms. Kate Goode for insulinoma membrane preparation.

This work was supported by grants from the British Diabetic Association and the Wellcome Trust.

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