MHC ANTIGEN INDUCTION BY INTERFERON γ ON CULTURED MOUSE PANCREATIC β CELLS AND MACROPHAGES

Genetic Analysis of Strain Differences and Discovery of an "Occult" Class I-like Antigen in NOD/Lt Mice

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There is considerable interest concerning the pathological consequences of aberrant expression of HLA-DR (class II) MHC products on endocrine cells from patients with autoimmune diseases such as insulin-dependent diabetes mellitus (1). The significance of MHC gene expression in pancreatic islet cells remains controversial (2). Normally, pancreatic β cells of mice constitutively express low levels of class I, but do not express class II antigens (3). In Non-Obese Diabetic (NOD)[1] mice, which spontaneously develop autoimmune insulin-dependent diabetes, there have been conflicting reports as to whether I-A antigens are constitutively expressed on β cells (4, 5). Similar controversy exists for other inbred strains. The situation in situ is complicated by the finding that intra-islet macrophages (Mϕ) phagocytosing β cell cytoplasm stain positive for both insulin and I-A (6). Diabetes induced by multi-dose streptozotocin in CBA/Wehi (7) and C57BL/KsJ (8) male mice is associated with an increase in cells expressing class II MHC antigens on their surfaces; in the case of C57BL/KsJ mice, infiltrating Mϕ and not β cells comprise the $I\text{-}A^+$ cells (8). Momburg et al. (9) did not observe I-A antigens on islets from B10.BR mice after in vivo treatment with IFN-γ. However, Wright et al. (10) reported induction of $I\text{-}A^k$ on free-floating islet preparations from B10.BR ($H\text{-}2^k$) donors cultured in the presence of IFN-γ. Islet cell monolayers from CBA/Wehi (also $H\text{-}2^k$) cultured in the presence of high concentrations of IFN-γ did not express class II unless TNF-α was also included (11). In this report, we show that gene(s) in the CBA/J inbred strain background exert *trans*-suppressive control over islet-specific class II gene expression in the diabetes-susceptible NOD inbred background. In the course of these studies, an unexpected IFN-γ inducible ("occult") expression of a class I-like cell surface antigen was also discovered that may be of potential pathogenetic significance in the autoimmune diabetes of NOD/Lt mice.

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[1] *Abbreviations used in this paper:* CI, cytotoxic index; NOD, Non-Obese Diabetic.

Materials and Methods

Mice. Non-Obese Diabetic (NOD/Lt) and Non-Obese Normal (NON/Lt) mice, including hybrid crosses, were bred in our research colony from breeding pairs kindly provided by Dr. Masakuzu Hattori (Joslin Diabetes Center, Boston, MA). CBA/J were obtained from the Animal Resources Unit of The Jackson Laboratory. All mice were maintained in a specific pathogen-free facility on a 14:10 light/dark cycle, and allowed free access to food (diet 96W; Emory Morse Co., Guilford, CT) and chlorinated drinking water.

mAbs and Antisera. Both NOD and NON mice possess recombinant MHC haplotypes that appear unique and to which formal haplotype designations have not yet been assigned. The *H-2* complex of NOD is K^d $I\text{-}A^{nod}$ $I\text{-}E^o$ D^b; that of NON is K^b $I\text{-}A^{non}$ $I\text{-}E^{k\text{-}like}$ D^b (12). CBA/J mice have a standard $H\text{-}2^k$ haplotype. The 28-13-3 mAb (13) was used at a 1/4,000 dilution to detect H-2K^b on NON/Lt cells and an IFN-γ-inducible crossreactive class I-like antigen on NOD/Lt cells in culture. This mAb defines public specificity H-2.39, an allodeterminant in the second (C1) external domain shaped by the $H\text{-}2K^b$ and $H\text{-}2K^f$ haplotypes (14). The 31-3-4 mAb (15) was used at a 1/5 dilution to detect H-2K^d expressed on NOD/Lt cells, and mAb 11-4.1 (15) was used at a 1/100 dilution to detect H-2K^k on CBA/J cells. For detection of class II MHC antigens, mAb 26-7-11S (12) was used at a dilution of 1/80 to detect I-A^k on CBA/J cells. A.TH anti-A.TL (I-A^s anti-I-A^k, broadly crossreactive with most standard class II haplotypes except *s*) was purchased from Accurate Chemical & Scientific Corp. (Westbury, NY) and used at a dilution of 1/15. mAb 28-13-3 and 11-4.1 were used as ascites; 31-3-4S and 26-7-11S were used as culture supernatants. For detection of the unique class II molecules of NOD and NON, AS-3 (anti-I-A^{NOD}) and AS-4 (anti-I-A^{NON}) alloantisera were produced in our laboratory; both exhibited titers greater than 1/400 in microcytotoxicity assay. AS-3 was produced by immunizing (NON/Lt × C3H.HTG/SnJ)F$_1$ mice with irradiated NOD/Lt splenocytes and used at a dilution of 1/100. Immunoprecipitation studies demonstrated that AS-3 was specific for NOD I-A (personal communication, Dr. H. O. McDevitt, Stanford University Palo Alto, CA). AS-4 was produced by immunizing (NOD/Lt × B6.PL-*Thy-1*^{a})F$_1$ mice with irradiated NON/Lt splenocytes and was also used at a 1/100 dilution. Splenic leukocytes from NOD/Lt, NON/Lt, and (NOD × NON)F$_1$ mice were phenotyped for cell surface H-2K antigen expression using a FACSCAN cytofluorograph as described previously (16). In brief, freshly obtained splenocytes were incubated with or without primary mAbs 28-13-3 (for H-2K^b) and 31-3-4S (for H-2K^d) for 30 min at 4°C. Cells were washed and incubated another 30 min at 4°C with fluorescein-labeled goat-anti-mouse IgG (GAM). Cells were stained with 40 μg/ml propidium iodide for analysis of viability, and dead cells were excluded from the analysis. Splenic B lymphocytes express surface Igs that bind the second antibody (GAM). Therefore an aliquot of cells from each genotype was stained with GAM only (no primary mAb) such that positive staining B lymphocytes in each population (Fig. 2, *left panels*) would be subtracted as background from the staining obtained when primary mAb was present (Fig. 2, *middle and right panels*).

Mϕ and Islet Cell Cultures. Peritoneal Mϕ were thioglycolate recruited and harvested as previously described (17). Mϕ were seeded at 5 × 10^5 cells/ml/well of 24-well plates (Corning) in DME supplemented with 16.5 mM glucose, 0.02% BSA, MEM nonessential amino acids, 44 mM sodium bicarbonate, 15 mM HEPES, 50 mg/liter gentamicin sulfate, 10% heat-inactivated FCS, with or without 50 U/ml rat IFN-γ, kindly provided by Dr. Peter van de Meide, T.N.O. Primate Center, Rijswijk, Netherlands. The peritoneal Mϕ were cultured in the presence or absence of IFN-γ for 3 d unless otherwise specified. All tissue culture reagents were obtained from Gibco Laboratories, Grand Island, NY.

Pancreatic islets were isolated for culture by collagenase digestion as described previously (18). Whereas CBA/J and NON/Lt islet donors of all ages were used, only young aglycosuric (6–8-wk-old) NOD/Lt islet donors were used. Hand picked islets were seeded 30/well in 24 well plates and incubated in 1 ml of DME supplemented as described above. Pancreases from four to five donors per genotype were used per isolation and the islets pooled (~150 islets picked from each pancreatic digest). After an initial 3-d period in serum-containing medium during which islet attachment and spreading of cells occurred, serum was omitted for one or two refeedings as required to inhibit fibroblast growth, with refeeding every 3–4 d until islets had monolayered (14 d). At day 14, the islet monolayers were refed with medium with

or without 50 U/ml IFN-γ for 6 d. Cultures were then tested for expression of MHC antigens using the chromium-release assay described below.

Genetic Analysis of "Occult" Gene Expression. To determine chromosomal localization of a IFN-inducible crossreactive epitope in NOD/Lt detected by mAb 28-13-3, and suppressed by the CBA/J inbred background, reciprocal (NOD/Lt × CBA/J)F_1 hybrids were produced and peritoneal Mϕ response to IFN-γ induction of the "occult" locus analyzed. F_1 mice were backcrossed to both the NOD/Lt and CBA/J parental strains, and peritoneal Mϕ were isolated from 8–12-wk-old first backcross (BC1) progeny. After culture for 3 d in the presence of 50 U/ml IFN-γ, reactivity of these peritoneal Mϕ with mAb 28-13-3, 31-3-4, and 11-4.1 was assessed using the ^{51}Cr-release assay described below. The latter two monoclonals permitted MHC phenotyping; in the backcross to NOD/Lt, half of the segregants would theoretically be *H-2K^d* homozygotes and half *H-2K^d/H-2K^k* heterozygotes, while in the backcross to CBA/J, half would be *H-2K^k* homozygotes and half would be *H-2K^d/H-2K^k* heterozygotes.

Chromium-release Assays. Cells to be assayed by complement mediated lysis were incubated at 37°C for 90 min in 250 μl of DME containing 10 μCi/m ^{51}Cr (ICN Biomedical, Inc., Irvine, CA). Cells were then rinsed three times with HBSS + 0.1% BSA. Triplicate cultures were incubated at 4°C with and without diluted antibodies for 30 min followed by incubation at 37°C for 3 h with rabbit complement (Low-Tox-M; Accurate Chemical & Scientific Corp., Westbury, NY) diluted 1/12. Supernatants were removed to determine radioactivity (cpm) released. Cells remaining were solubilized in 1% SDS and absorbed with cotton swabs to determine unreleased radioactivity. Percent release was determined from the equation [cpm in medium/(cpm in medium + cpm in cells)] × 100%. The cytotoxic index (CI) was obtained from the equation CI = 100% × {[(percent release of antibody + complement) − (percent release of complement alone)]/[100% − percent release of complement alone]}.

H-2K^b Alloreactive NOD T Cells. A model system was designed to test the question as to whether IFN-γ-induced expression of the "occult" antigen on NOD/Lt β cells could be recognized by NOD alloreactive T cells. Four 6-wk-old NOD/Lt females (*H-2K^d*) received weekly tail vein injections (over 5 wk) of 10^7 irradiated splenocytes (2,000 rad, ^{137}Cs source) from an incipient NOD.NON-*H-2K^b* congenic stock (N_6F_1). Three unmanipulated NOD/Lt females served as unprimed controls. T cells were nylon wool purified (16) from splenocytes of primed and unprimed 11-wk-old NOD females, and 5 × 10^5 of them were cocultured in triplicate in flat-bottomed 96-well microtiter plates (Corning) containing 12 islets/well from NOD.NON-*H-2K^b* (positive controls) or standard (*H-2K^d*) NOD donors. The islets were pretreated with or without 50 U/ml IFN-γ for the 6 d before coculture with T cells. The islet-T cell cocultures were incubated for 4 d at 37°C and labeled with 0.5 μC/well [^{3}H]thymidine (New England Nuclear, Boston, MA) over the final 8 h of incubation. The cultures were then harvested onto glass fiber disks with a PHD cell harvester (Cambridge Technology, Cambridge, MA), and the disks were counted in 3 ml of Aquasol-2 (New England Nuclear). Data are represented as Δcpm (the mean cpm of triplicate T cell cultures in the presence of islets minus the mean response of T cells and islets cultured separately in medium only). In a separate experiment, 10^6 T cells isolated from NOD.NON-*H-2K^b* primed NOD mice were maintained for 2 wk on IFN-γ-pretreated NOD islets in medium containing 10% Con A-conditioned culture supernatant as a source of exogenous cytokines. The T cells were then harvested and seeded in triplicate at 10^5/well in a 24-well cluster plate containing islet monolayers from NOD.NON-*H-2K^b* or NOD mice that had been precultured with and without IFN-γ before labeling with ^{51}Cr for 4 h. T cell mediated lysis (CML) was assessed by ^{51}Cr release over a subsequent 4-h incubation period as described above.

Results

IFN-γ-inducible Class II MHC In Islets and Mϕ. As illustrated in Table I, levels of constitutive and IFN-γ-inducible cell surface expression of class II antigens depended upon type of cells cultured (islet cells vs. peritoneal Mϕ) and inbred strain background (CBA/J, NON/Lt, NOD/Lt, and F_1 hybrids). The CBA/J inbred strain background provided the most striking example of tissue-specific differences in IFN-γ-regulation of I-A antigen expression. In the absence of IFN-γ in the culture medium,

TABLE I

IFN-γ Induction of MHC Class II in Cultured Islet Monolayers and Cultured Peritoneal Mϕ

Strain	IFN-γ	Cytotoxic index							
		Islets				Macrophages			
		$I\text{-}A^k$	$I\text{-}A^{NON}$	$I\text{-}A^{NOD}$	I-A*	$I\text{-}A^k$	$I\text{-}A^{NON}$	$I\text{-}A^{NOD}$	I-A*
CBA/J	–	0	0	0	2	2	0	0	0
	+	0	4	0	1	19	0	0	32
NON/Lt	–	0	16	0	0	0	7	4	4
	+	0	17	0	7	0	40	6	32
NOD/Lt	–	0	0	0	0	0	0	51	ND‡
	+	0	0	11	9	0	0	71	ND
(CBA × NOD)F_1	–	0	0	1	0	0	0	25	0
	+	0	0	0	0	25	0	41	43
(NON × NOD)F_1	–	ND	0	0	0	0	6	49	6
	+	ND	11	21	19	0	40	51	46

* A.TH anti A.TL antiserum, broadly cross-reactive.

‡ Indicates values that have not been determined. Cytotoxic indices were obtained from antibody plus complement-mediated ^{51}Cr-release assays using the mAb or I-A alloantisera reagents described in Materials and Methods. Triplicate wells containing islet cells or Mϕ cultured in the presence or absence of IFN were treated with complement alone or complement plus antibody and cytotoxic index determined as described in the text.

neither CBA/J islet monolayers cultured for 21 d nor Mϕ cultured for 3 d expressed significant surface $I\text{-}A^k$ [CI = 0 (CBA/J islets) and 2 (CBA/J Mϕ) using the anti-$I\text{-}A^k$ mAb]. IFN-γ elicited $I\text{-}A^k$ expression on the surfaces of cultured CBA/J Mϕ (CI = 19), but not on cultured CBA/J islet cells (CI = 0), indicating a tissue-specific difference in IFN-γ regulation of I-A antigen expression within the same inbred strain.

The importance of regulatory genes in the inbred strain background in determining tissue-specific as well as strain-specific responsiveness to IFN-γ was further underscored when the NON and NOD backgrounds were studied (Table I). In contrast to cultured CBA/J Mϕ, which exhibited IFN-γ-inducible, but virtually no constitutive $I\text{-}A^k$ expression, cultured NON/Lt and NOD/Lt Mϕ expressed $I\text{-}A^{NON}$ and $I\text{-}A^{NOD}$ class II antigens constitutively. However, a striking difference in level of constitutive expression between NON/Lt Mϕ (CI = 7) and NOD/Lt Mϕ (CI = 51) was observed (Table I). As was the case with CBA/J Mϕ, I-A expression in NOD/Lt and NON/Lt Mϕ was IFN-γ inducible (CI = 40 and 71, respectively). Although the CI of cultured NON/Lt Mϕ was strongly increased by IFN-γ, the induced level was lower than that level constitutively expressed by NOD/Lt Mϕ. On cultured NON/Lt islet cells, constitutive $I\text{-}A^{NON}$ expression (CI = 16) was observed that was not significantly elevated by IFN-γ (CI = 17). Again, islet cultures from NOD/Lt differed from NON/Lt. Although $I\text{-}A^{NOD}$ was inducible on cultured NOD/Lt islet cell surfaces (CI = 11), it was not constitutively expressed (Table I).

Tissue-specific differences in IFN-γ responsiveness within the same inbred strain were again observed when surface $I\text{-}A^{NOD}$ was compared on NOD/Lt islet cells versus Mϕ (Table I). Although $I\text{-}A^{NOD}$ was inducible on the cultured islet cell surfaces, it was not constitutively expressed. In contrast, NOD/Lt Mϕ cultures were

constitutively expressing high levels that were only moderately elevated further by IFN-γ (Table I). When Mϕ cultures from (NON × NOD)F_1 hybrids were screened for expression of each parentally derived I-A antigen, both parental patterns of constitutive and IFN-γ-inducible expression were maintained. Thus, in Mϕ cultured from the (NON × NOD)F_1 hybrid, I-A^{NON} was constitutively low, but strongly IFN-γ inducible as in the NON/Lt parental strain (Table I). Concordant with the NOD/Lt parental strain, a very high constitutive expression of I-A^{NOD} was observed in Mϕ cultured from the F_1 hybrids (Table I). Hence, differences in independent and apparently *cis*-active IFN-γ response elements controlling *I-A* gene expression distinguished the NOD/Lt and NON/Lt inbred strain backgrounds.

Whereas *cis*-active, IFN-γ-sensitive regulatory elements of class II structural genes were independently operative in Mϕ of (NON × NOD)F_1 hybrids, a completely different regulation was observed in islet, but not Mϕ cultures established from (CBA × NOD)F_1 mice. As already described, parental CBA/J islet cells did not constitutively express I-A^k and expression was not IFN-γ inducible, whereas parental NOD/Lt islet expression of I-A^{NOD} was not constitutive, but was IFN-γ inducible. In islet cells from the F_1 hybrids between these two strains, the CBA/J genome interacted in a trans-fashion with the NOD/Lt genome to suppress IFN-γ inducibility of I-A^{NOD} (Table I). Interestingly, this *trans*-suppressive action of the CBA/J genome on expression of I-A^{NOD} was islet cell specific, since no similar *trans*-suppression was observed in the cultured Mϕ from the F_1 hybrids (Table I).

All ^{51}Cr-release data generated by cytotoxicity assays were confirmed by visual inspection under phase microscopy of the cultured cells undergoing antibody plus complement-mediated lysis. At the morphological level, the complete resistance of IFN-γ-treated CBA/J islet monolayers to lysis by anti-I-A^k plus complement contrasted to IFN-γ-treated NOD/Lt monolayers. The latter exhibited clear lysis in the presence of anti-I-A^{NOD} plus complement (Fig. 1). Previous ultrastructural analysis of these monolayer islet cell cultures has established that the majority of the cells (>70%) are glucose-responsive β cells (19); intraislet macrophages/dendritic cells comprise only about 4% or fewer of the cells in these islet monolayers such that the majority of the cells in Fig. 1 *D* undergoing extensive antibody plus complement-mediated cytolysis probably are β cells. Thus, IFN-γ inducibility of class II antigen on mouse β cell surfaces is strongly dependent upon the genetic background of the islet donor strain, with *trans*-suppressive factor(s) in the CBA/J genome apparently underlying nonresponsiveness to IFN-γ.

IFN-γ-inducible Class I MHC Gene Expression In Islets and Mϕ. Table II compares the constitutive and IFN-γ-inducible cell surface expression of MHC class I antigens as a function of cell type (islet cell vs. peritoneal Mϕ) and inbred strain background (CBA/J, NON/Lt, NOD/Lt, and F_1 hybrids). Without IFN-γ in the culture medium, CBA/J Mϕ expressed no detectable K^k after 3 d in culture, whereas expression was maintained in its presence (CI = 46). In contrast, Mϕ from both NOD/Lt (K^d) and NON/Lt (K^b) maintained high constitutive levels of class I expression (CI = 66 and 71, respectively) following 3 d of culture in the absence of IFN-γ, with only a slight increase in expression in its presence (CI = 70 and 81, respectively). Crossing CBA/J (nonconstitutive for class I expression on Mϕ after 3 d in vitro) and NOD/Lt (constitutively expressing after 3 d in vitro) produced F1 Mϕ that failed to constitutively express K^k in vitro while continuing to express

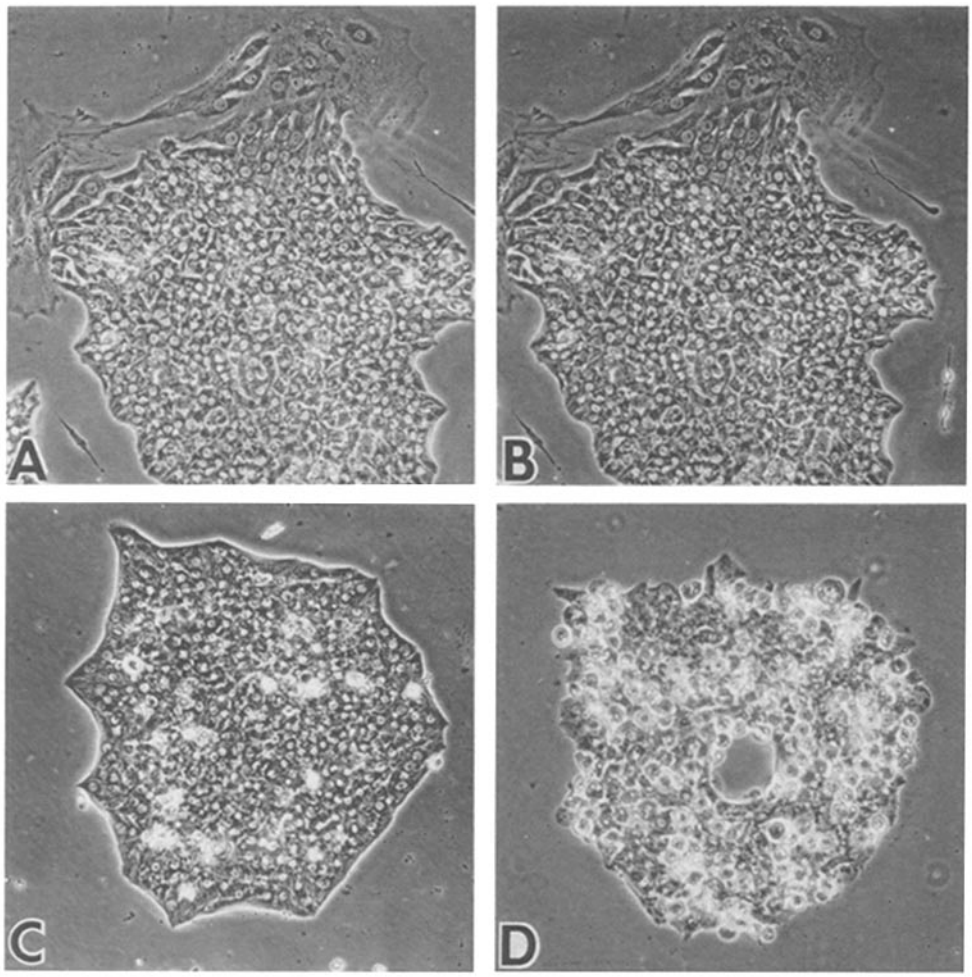

FIGURE 1. Morphological demonstration of IFN-γ-induced class II MHC induction on NOD/Lt, but not CBA/J islet cell monolayers. No cytolysis observed in CBA/J ($I\text{-}A^k$) islet monolayer before (*A*) and after (*B*) treatment with cytolytic mAb 26-7-11S (anti-$I\text{-}A^k$, CI = 0), and an intact NOD/Lt islet monolayer before (*C*) and extensive cytolysis in the same monolayer after (*D*) treatment with cytolytic alloantiserum AS-3 (anti-$I\text{-}A^{NOD}$, CI = 9.2).

K^d constitutively (CI = 45). This contrasted with the (NON × NOD)F_1 hybrids; the pattern of class I expression on Mϕ from these mice was identical to the parental strains with high constitutive levels of class I and little further induction by IFN-γ treatment (Table II). Thus, inbred strain-dependent factors regulating expression of MHC class I gene products on Mϕ in culture were *cis*-acting.

The pattern of expression of MHC class I surface antigens on long-term cultured islet cells as a function of inbred strain background was concordant to expression of the same antigens on cultured Mϕ from the same genotypes (Table II). CBA/J islet monolayers after 3 wk of culture did not constitutively express K^k, but expression was IFN-γ inducible (CI = 32). Class I antigens were constitutively expressed

TABLE II
IFN-γ Induction of MHC Class I Antigens in Cultured Islet Monolayers and Cultured Peritoneal Mϕ

		Cytotoxic index					
		Islets			Macrophages		
Strain (H-2K)	IFN-γ	H-2K^k	H-2^b	H-2^d	H-2K^k	H-2K^b	H-2K^d
CBA/J	–	0	1	2	0	0	0
(K^k)	+	32	1	0	46	0	0
NON/Lt	–	0	5	1	0	71	0
(K^b)	+	0	51	0	2	81	0
NOD/Lt	–	0	0	16	0	0	66
(K^d)	+	0	27	39	0	43	70
(CBA × NOD)F$_1$	–	0	0	18	0	0	45
(K^k/K^d)	+	42	0	40	41	6	56
(NON × NOD)F$_1$	–	ND*	17	19	0	64	64
(K^b/K^d)	+	ND*	48	44	0	70	68

* Indicates values that have not been determined. Cytotoxic indices were obtained from antibody plus complement-mediated ^{51}Cr release assays as described in Materials and Methods.

and were IFN-γ inducible on islet cultures from NOD/Lt and NON/Lt. The levels of constitutive and IFN-γ–inducible class I antigen expression on islet monolayers were lower than observed for Mϕ of the same genotype, suggesting tissue specific differences in antigen density. Again, F$_1$ hybrids between the nonconstitutively expressing CBA/J and constitutively expressing NOD/Lt showed *cis*-acting regulation in that the CBA/J *H-2K^k* gene in islet cells from F$_1$ donors was repressed in the absence of IFN-γ whereas the NOD/Lt *H-2K^d* gene was constitutively expressed in the absence of IFN-γ and further inducible in its presence.

Demonstration of an "Occult" Class I-like antigen on NOD/Lt Islets and Mϕ. In performing the analyses depicted in Table II, monolayer cultures of NOD/Lt and CBA/J islet cells and Mϕ were tested for reactivity with mAb 28-13-3 as well as other antibodies that should have been unreactive (i.e., "irrelevant" mAb). As expected, CBA/J cells were completely unreactive to mAb 28-13-3 (Table II). Further, as confirmed by the cytofluorographic analysis shown in Fig. 2, freshly prepared splenic leukocytes from NON/Lt (*K^b*) or from (NOD × NON)F$_1$ (*K^b/K^d*) mice, but not from NOD/Lt (*K^d*) mice, specifically bound mAb 28-13-3. Therefore, the finding that mAb 28-13-3 plus complement was nontoxic to either NOD/Lt islet cells or Mϕ cultured in the absence of IFN-γ (CI = 0) was expected. Unexpectedly, as documented in Table II, IFN-γ treatment induced a latent reactivity to mAb 28-13-3 (CI = 27), indicating induction of a K^b-like "occult" crossreactive antigen presumably mimetic with public specificity H-2.39. FACS analysis was repeated using a biotinylated preparation of mAb 28-13-3 followed by addition of a FITC-avidin conjugated in an attempt to see if the cross-reactive epitope recognized by this antibody could be observed in the absence of the high B lymphocyte background produced when a FITC-labeled goat anti-mouse antibody detection system was used with unlabeled mAb 28-13-3 as a primary antibody. While 86.7% of NON/Lt splenocytes bound

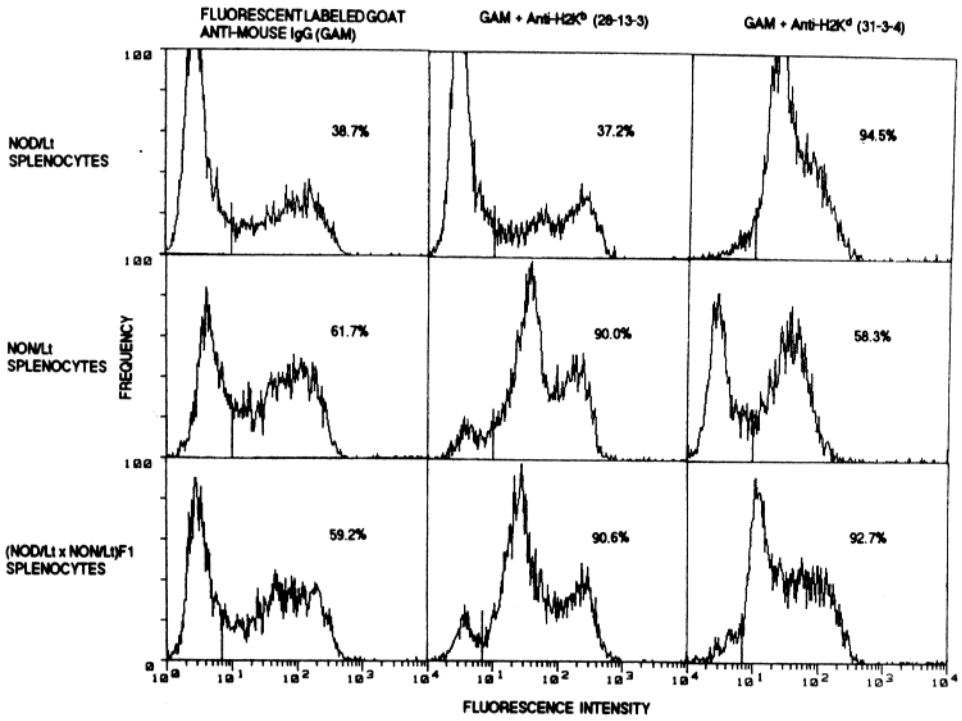

FIGURE 2. FACS analysis of NOD/Lt, NON/Lt, and (NOD/Lt × NON/Lt) splenic leukocytes showing specific staining (above GAM-only stained controls) of uncultured NON/Lt, and (NOD/Lt × NON/Lt), but not NOD/Lt cells by mAb 28-13-3. The percentages indicate the number of positively staining cells to the right of the gated threshold (vertical line).

the biotinylated reagent, no binding beyond the FITC-avidin control was observed in freshly-isolated NOD/Lt splenocytes (data not shown).

The (CBA × NOD)F_1 hybrid provided insight into the regulation of this "occult" gene. As shown in the results presented in Table I and described above, the *trans*-active factor(s) emanating from the CBA/J genome were capable of suppressing I-A^{NOD} expression only in (CBA × NOD)F_1 islet cell cultures, but not in F_1 Mϕ cultures (Table I). Therefore, it was not anticipated that the *trans*-active factor produced from the CBA/J genome would suppress the "occult" crossreactive entity in both islet and Mϕ cultures established from (CBA × NOD)F_1 hybrids. Yet this dual suppression in both cell types was indeed observed (Table II). Therefore, the "occult" locus appeared to be independently regulated from the standard class I and II loci.

Time Course of IFN-γ Induction of "Occult" vs. Class I and II Antigens. The time course of IFN-γ induction of the occult antigen as detected by mAb 28-13-3 versus the induction curve for the standard MHC class I H-$2K^d$ detected by mAb 31-3-4 is shown for peritoneal Mϕ in Fig. 3. These curves clearly established the separate regulation of the two antigens; the H-$2K^d$ antigen was constitutively expressed on Mϕ, and higher levels were induced by IFN-γ. Compared with I-A^{NOD}, which had been significantly induced by 16 h, the "occult" antigen required between 48 and 64 h to be expressed on the cell surfaces. After prolonged culture after removal of

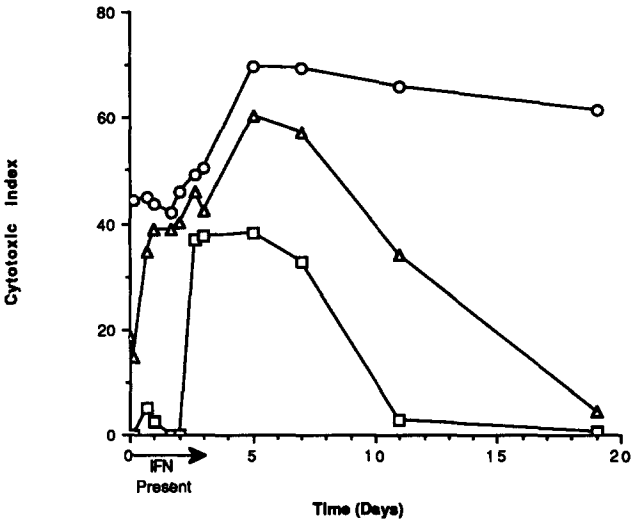

FIGURE 3. Time course of surface antigen induction and decay on NOD/Lt Mϕ after a 3-d IFN-γ pulse. NOD/Lt peritoneal Mϕ treated in vitro with 50 U/ml IFN-γ for 3 d, rinsed, refed, and incubated without IFN-γ for up to 16 d, were assayed with antibody plus complement-mediated ^{51}Cr release at various time points for I-A^{NOD} (Δ). H-2K^d (O), and "occult" antigen (□) expression.

IFN-γ at 72 h, the differential decay rate of the constitutively expressed K^d versus the nonconstitutive but IFN-γ-inducible occult antigen is evident. Thus, the occult, but not the K^d antigen expression in vitro required the continued presence of IFN-γ. Although the induction rates for occult antigen vs. I-A^{NOD} were clearly distinguishable, the induced levels of both antigens appeared to remain stable for ~4 d, and the subsequent decay rates for both were quite similar (Fig. 3). However, I-A^{NOD} was induced by IFN-γ to a higher level than was the occult antigen such that by culture day 11 (8 d after IFN-γ withdrawal), occult expression had declined to essentially baseline whereas I-A^{NOD} was still strongly expressed (Fig. 3).

Genetics of Occult Gene Expression on Mϕ. As established in Table II, *trans*-acting regulatory elements in the CBA/J inbred strain background were capable of suppressing IFN-γ-stimulated occult antigen expression in both islet cell and Mϕ cultures from (CBA × NOD)F_1 donors. Accordingly, the number of genes and their potential chromosomal localization could be analyzed by backcrossing IFN-γ-noninducible F_1 hybrids to both the NOD/Lt (inducible) and CBA/J (noninducible) parental strains, and testing BC1 segregants for IFN-γ-inducibility of occult antigen. The results of ^{51}Cr-release assay using mAb 28-13-3 plus complement are presented in Fig. 4 and clearly establish that the structural gene for the occult locus is tightly linked to the MHC of NOD mice (Chr 17). The data obtained from backcross to NOD/Lt further indicate that *trans*-suppressors contributed by the CBA/J genome may be under polygenic control. Chromium-release assay produced a mean CI of 43 for IFN-γ-cultured NOD/Lt Mϕ versus a mean CI of 1.1 for CBA/J and CI of 6 for the F_1. Mice backcrossed to NOD/Lt (D/BC1) and CBA/J (C/BC1) were phenotyped with mAbs for H-2K antigen, and this was correlated with the level of occult crossreactivity with 28-13-3. D/BC1 mice homozygous for the NOD Chr 17 marker (K^d) had a high NOD/Lt-like value (mean CI = 43.9); interestingly, 5/19 of this group had a high intermediate level of expression, suggesting that at least one other co-dominant regulatory locus was segregating (Fig. 4). IFN-γ-treated Mϕ from D/BC1 mice heterozygous at the H-2K locus (K^k/K^d) exhibited a range of

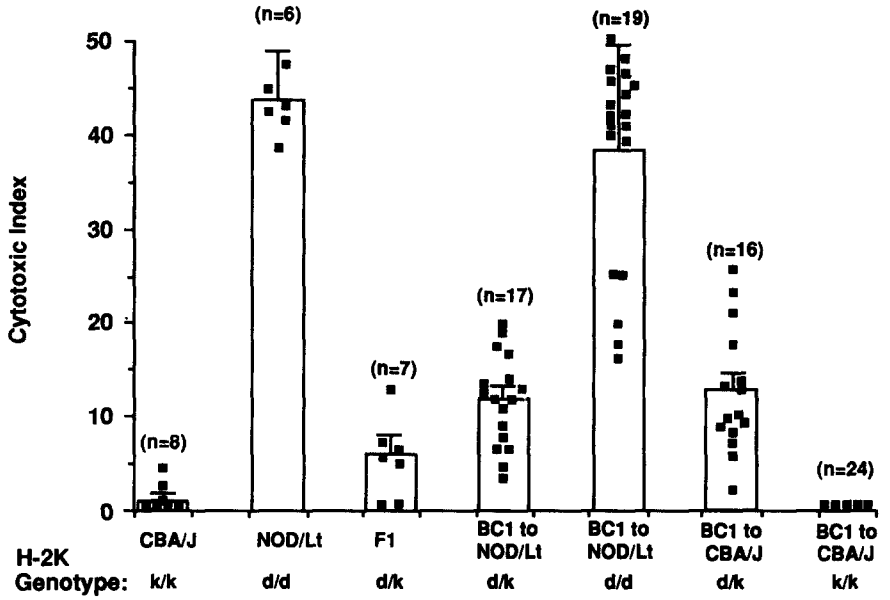

FIGURE 4. Genetics of occult gene expression on peritoneal Mϕ from first backcross mice to NOD (D/BC1) or CBA (C/BC1) showing linkage of the structural locus to Chr 17 of NOD. Complement + mAb 28-13-3 (anti-H-2K^b)-mediated ^{51}Cr release was used to determine the varying levels of occult antigen expression induced on the surface of Mϕ treated for 3 d with 50 U/ml IFN-γ.

values from intermediate, to low F_1-like values (mean CI = 11.8), further indicating linkage of the structural locus for the occult antigen to the MHC of NOD. The localization of the occult locus to Chr 17 of NOD/Lt was confirmed in C/BC1 mice since no Mϕ cultures derived from the K^k homozygous segregants expressed IFN-γ-inducible occult antigen. Occult expression was observed to varying degrees in C/BC1 mice that were heterozygous at the H-2K locus. The mean CI of 12.9 in Mϕ isolated from these mice was almost the same as that observed in the K^d/K^k heterozygous D/BC1 segregants (Fig. 4). Although the occult-suppressive nature of CBA/J modifiers in the F_1 suggested that they were dominant in action, the wide variation in CI values in the MHC heterozygous D/BC1 and C/BC1 segregants (from low "F_1"-like to high intermediate levels) suggested that CBA-mediated suppression was polygenic in nature. That the CI in K^d/K^k heterozygotes was usually considerably less than 50% of that observed in K^d/K^d homozygotes suggested that more than an occult structural gene dosage effect was involved.

Since Mϕ harvesting from BC1 mice was a nonlethal procedure, the Mϕ cultures could be used to MHC-type the mice and establish their level of occult inducibility. From this characterized segregant population, representative individuals were killed, and their pancreatic islets were isolated and placed into culture. Levels of occult expression on these islet monolayers treated with IFN-γ could thus be compared with the pervious data obtained from Mϕ from the same mouse. As shown by the morphological data presented in Fig. 5, the CI for occult antigen expression generally corroborated the data obtained for Mϕ from the same mice. As shown in Table I, maximal CI from IFN-γ-cultured NOD/Lt islets in response to mAb 28-13-3 during

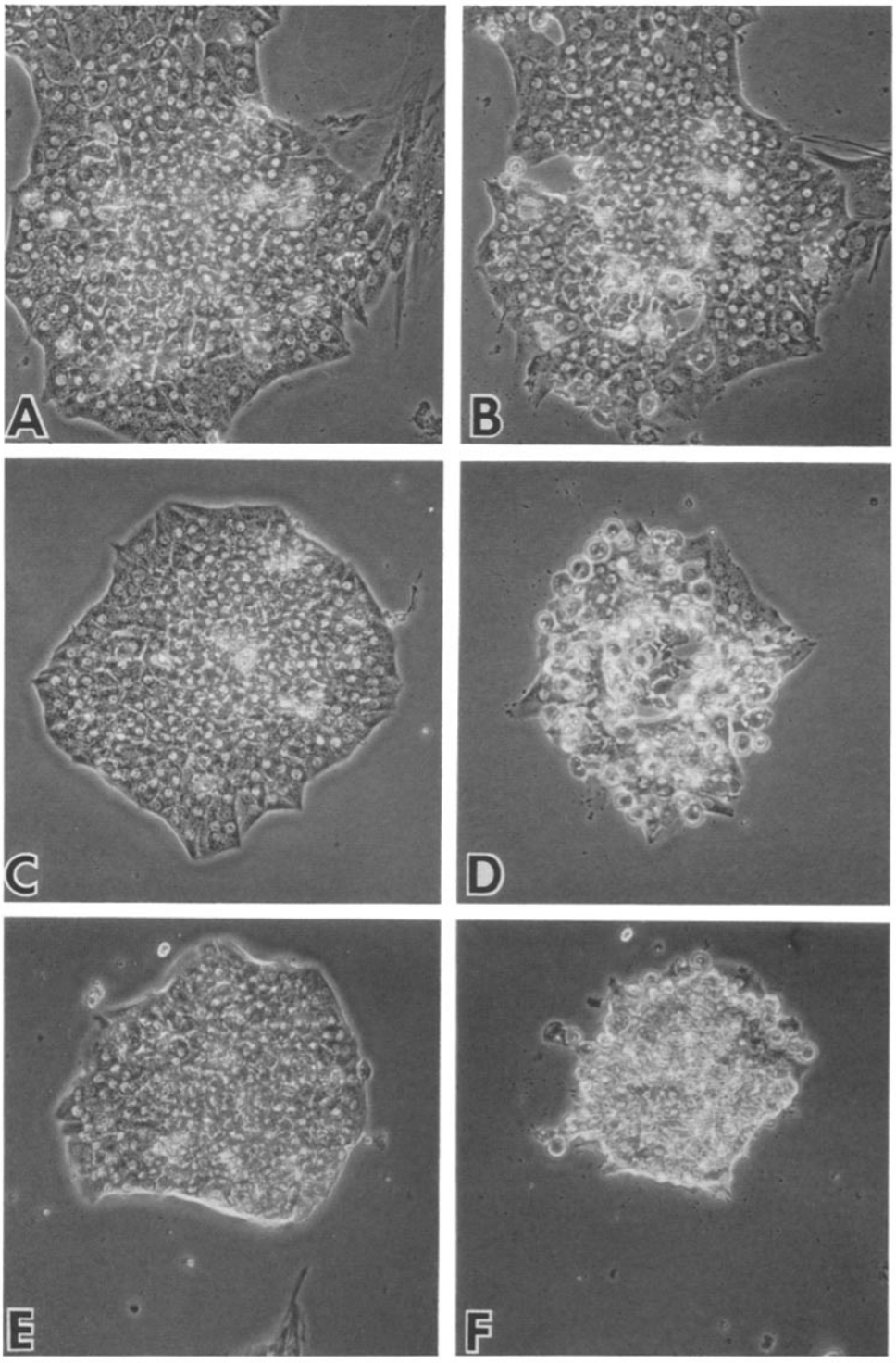

FIGURE 5. [(CBA/J × NOD/Lt)F_1 × NOD/Lt]BC1 islet monolayers before and after treatment with mAb 28-13-3 (anti-H-2K^b) and complement in an assay for occult antigen expression. (*A* and *B*). Before and after treatment of an islet monolayer from a K^d/K^k MHC heterozygote who had previously donated Mϕ yielding a low CI of 7.0 (i.e., *trans*-suppressed for occult). There is visual evidence of only slight islet cell destruction, as confirmed by an islet CI of 4.2. (*C* and *D*). Before and after treatment of an islet monolayer from a K^d/K^k MHC heterozygote who had previously donated Mϕ yielding an intermediate CI of 17.4 (i.e., incomplete *trans*-suppression of occult). Clear visual evidence of islet cell destruction, confirming an islet CI of 13.2, is evident. (*E* and *F*). Before and after treatment of an islet monolayer from a K^d/K^d MHC homozygote who had previously donated Mϕ yielding an high NOD/Lt-like CI of 44.6 (i.e., no *trans*-suppression of occult). Clear visual evidence of islet cell destruction, confirming an islet CI of 16.4, is again evident.

a 3-h lysis period was only 27 in comparison to a CI of 43 from Mϕ over the same time period. Interestingly, the lower CI for islets appeared to represent an inherent property of the islet cultures. As shown in Fig. 5, more visual evidence of islet cell destruction was documented by phase microscopy of islets from a K^d homozygous donor (whose Mϕ exhibited a CI of 44.6) than from a K^d/K^k) heterozygous donor (whose Mϕ yielded a CI of 17.4). Yet when compared with a CI difference of 27.2 between the two Mϕ populations, only a 3.1% difference in CI was observed between the two islet populations depicted (Fig. 5 *D* vs. *F*), despite the visual evidence of greater lysis from the K^d homozygous cell population.

Can T Cells Recognize Occult Antigen on NOD/Lt Islet Cells? Expression of the occult antigen on NOD/Lt pancreatic β cells could play a role in disease pathogenesis if recognized by T cells. This possibility was investigated by examining the ability of T cells from NOD/Lt mice to respond to IFN-γ-treated NOD/Lt islets following priming with splenocytes from an incipient NOD/Lt congenic stock homozygous for $H\text{-}2K^b$ (and thus expressing public specificity H-2.39 that is mimetic with the occult antigen). As shown in Fig. 6, T cells from NOD mice that had been primed to $H\text{-}2K^b$ only responded blastogenically to NOD/Lt islets that had been precultured with IFN-γ, but not NOD/Lt islets precultured in medium only. The primed T cell population also responded blastogenically to K^b antigen-expressing islets isolated from the congenic and precultured either in the presence or absence of IFN-γ. Both K^b antigen-primed and unprimed NOD/Lt T cells responded to congenic islets expressing the K^b antigen, indicating the congenic islets were capable of stimulating primary allogeneic responses. The response of unprimed NOD/Lt T cells to IFN-γ precultured NOD/Lt islets was similar to the K^b-primed NOD/Lt T cell response to NOD/Lt islets precultured in the absence of IFN-γ. Collectively, these results indicated that priming of NOD/Lt T cells to K^b resulted in a T cell population capable of responding blastogenically to NOD/Lt islets expressing the IFN-γ inducible occult antigen. In a separate experiment, K^b primed NOD/Lt T cells were incubated with IFN-γ pretreated NOD/Lt islets for 2 wk in the presence of Con A-conditioned CBA/J splenocyte culture supernatant as a source of exogenous help. T cells from this culture were then added at 10^5/well to triplicate cultures of ^{51}Cr-labeled NOD/Lt islets that had been pretreated in the presence or absence of IFN-γ. These T cells elicited a significantly higher ^{51}Cr release from

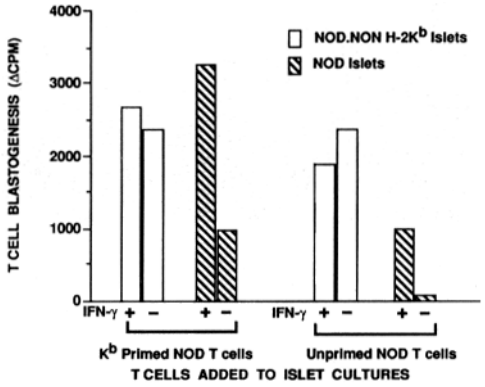

FIGURE 6. NOD.NON-$H\text{-}2K^b$ primed T cells respond blastogenically to IFN-γ precultured NOD islets. Nylon wool-purified T cells from unprimed NOD/Lt or NOD/Lt mice primed five times with splenocytes from NOD.NON-$H\text{-}2K^b$ congenic were added in triplicate at 5×10^5 to islets isolated from the congenic or standard NOD/Lt mice and precultured in the presence or absence of IFN-γ. The cultures were labeled with 0.5 μCi/well [^{3}H]thymidine over the final 8 h of a 4-d incubation.

NOD/Lt islets pretreated with IFN-γ (CI = 10.3 ± 0.9) than from islets precultured in medium only (CI = 0). Collectively, these results indicate that NOD/Lt T cells primed to K^b could recognize the occult antigen expressed on NOD/Lt islets.

Discussion

The unexpected discovery of a cytokine-inducible cell surface antigen on cultured NOD/Lt islet cells suggested a potential pathogenetic triggering mechanism to activate autoimmune T cells. One of the puzzling aspects of diabetes pathogenesis in NOD mice has been that pancreatic leukocytic infiltrates appear shortly after weaning (3–5 wk of age), but insulitis does not become severe enough to effect a major reduction in β cell mass and islet insulin content until after 12 wk (20). IFN-γ is a product of T cells. It is conceivable that when a critical mass of periinsular T cells and other leukocytes is accumulated, cytokines such as IFN-γ lead to induction of heretofore silent genes such as the one encoding the MHC-linked occult antigen described in this report. It is noteworthy in this regard that Janeway et al. have proposed the existence of a population of T cells (TCR1, expressing rearranged δ/γ T cell receptor chains) whose function may be surveillance of the integrity of epithelial cells (21). The major function proposed for activated TCR1 cells is the destruction of injured or otherwise altered epithelial cells, and there is evidence that recognition of, and response to class I–like MHC gene products may be integral to the activation cycle (21). The present study has demonstrated that NOD/Lt T cells primed to the H-$2K^b$ alloantigen were not stimulated by untreated islet cultures from standard NOD/Lt donors (*H-2K*^{d}). However, when replicate cultures of islets from the same donors had been precultured in IFN-γ to elicit occult antigen expression, the anti-H-$2K^b$ primed T cells were either stimulated to proliferate in one experiment (Fig. 6) or to mediate antiislet cytotoxicity in a second experiment. The implication of these model studies in vitro is that if high levels of occult were expressed in situ, an immunogenic response might be anticipated. Our studies further demonstrated that IFN-γ not only stimulated expression of the occult antigen on NOD/Lt β cells, but also class I and class II MHC molecules. If the occult antigen can be recognized by T cells expressing rearranged α,β TCR chains (TCR 2 as proposed in [21]), the induction of standard MHC molecules on the pancreatic β cell surface could serve as restriction elements for presentation of the occult antigen. Pujol-Borrell and Bottazzo have recently proposed that tolerance to rare (low concentration) epithelial cell autoantigens is maintained by lack of efficient autoantigen presentation (22). The presentation of the occult antigen to TCR 2 cells by self-MHC may thus occur in a manner similar to the presentation of minor histocompatability antigens. However, recent studies with *I-E*$\alpha^d\beta^b$–expressing transgenic islet cells suggest that class II MHC gene products on the surface of β cells imparts T cell anergy rather than activation (23). Alternatively, as discussed above, TCR1 T cells have been proposed to play a role in surveillance of epithelia by responding in a non-MHC–restricted fashion to MHC class I like genes induced on epithelial cell surfaces by viral infection or other injury (21). Activation of endogenous retroviruses in pancreatic β cells has been associated with the development of insulitis and diabetes in NOD mice (24). The activation of these endogenous viruses may result in IFN-γ synthesis with subsequent induction of the occult antigen on pancreatic β cells leading to their elimination by TCR1 cells.

The genetic analysis of occult gene expression in Mϕ and islets strongly suggested that the structural gene for this locus was MHC-linked. In mice, in addition to the 3–6 class I genes within the MHC complex on Chr 17 encoding the classical transplantation antigens, there are 20–30 distinct class I-like genes with open reading frames localized to the TL complex distal to the H-2L marker locus of the MHC (25). The TL complex is subdivided into two regions, *Qa2* and *TL*. Qa-2 is a serologically defined polypeptide primarily expressed on cells of hematopoietic origin; it has been established that the *Q7* locus within the *Qa2* region of the *TL* complex encodes both a membrane bound as well as a secreted polypeptide indistinguishable from the Qa-2 antigen (26, 27). The allodeterminant on H-2K^b molecules recognized by mAb 28-13-3 apparently resides in the second external (C1) domain (9); comparison of the gene sequence coding for this domain versus the sequence coding for the same domain in the *Q7^b* locus of C57BL/10 revealed 87% homology (only 37 of 276 nucleotides discordant [27]). Although NOD/Lt lymphocytes have not yet been typed to determine whether they are high level expressors of Qa-2, they have in common the *H-2D^b* allele associated with high expression in the C57BL/10J and C57BL/6J strains. Indeed, restriction fragment analysis of DNA using a *H-2K* probe that crosshybridizes with *Q7/Q9* has shown a *Q7^b/Q9^b* restriction fragment pattern in NOD identical to that of Qa-2 high expression C57BL/6J mice, but distinct from low-expressing BALB/c mice (Lund, T., Dept. of Immunology, University College, London; personal communication). The *Q7^b/Q9^b* genes both encode a soluble M_r 39,000 protein (27). Preliminary mAb 28-13-3 immunoprecipitation studies of [^{35}S]methionine-labeled NOD/Lt Mϕ lysates have been performed in our laboratory. Autoradiographic analysis of an SDS-PAGE gel showed an IFN-γ-inducible 35,000 M_r protein that potentially could represent a biosynthetic form of the occult antigen. The differences in molecular weights would suggest that the occult protein is not identical to the product of *Qa7^b*. However, enzymatic removal of two *N*-linked carbohydrate chains from *Qa7^b*- or *Qa9^b*-encoded polypeptides did yield a molecule of M_r ~34,000 (27) such that the occult antigen might represent a less glycosylated form. If so, an important distinction still differentiates occult antigen from the Qa-2 polypeptides, and that is the lack of expression of occult on unstimulated splenic leukocytes as assessed by FACS (Fig. 2).

The occult antigen described in this report may also represent gene products of the hematopoietic histocompatability-1 (*Hh-1*) locus that has been mapped to chromosome 17 in linkage with the *H-2D* locus (28). Unlike standard MHC antigens that are codominantly expressed, *trans*-acting regulatory elements suppress expression of Hh antigen in F_1 hybrids prepared between Hh antigen–expressing and nonexpressing parental strains. A *trans*-regulatory gene model was proposed where the presence of a regulatory sequence blocked expression of particular Hh determinants, while the absence of the regulatory sequence allowed for gene expression. The suppression of *Hh-1* gene expression in F_1 hybrids is proposed to play a role in the resistance of these hybrids to reconstitution with bone marrow from the parental strain expressing the Hh antigen. Thus, the regulation of *Hh-1* gene expression follows the same pattern as expression of the occult antigen. Since the *Hh-1* locus is apparently in tight linkage with *H-2D* it is tempting to speculate that *Hh-1* may be one of the class I genes that have been mapped to the *H-2D* locus in addition to the standard *H-2D* gene. Like the occult antigen in this report, the products of

these genes (*D2, D3,* and *D4*) have recently been demonstrated to be capable of being recognized by CTL (29). Other genes with low polymorphism, but with broad expression in epithelial tissues have been reported (30) such that the exact identity of the structural gene encoding the occult antigen remains to be determined.

The functions of the class I-like antigens encoded in the *Qa/TL* region remain obscure; expression of Qa-2 antigen has been reported on mouse preimplantation embryos up to blastocyst stage (31). Since not all mouse strains express the Qa-2 antigen, embryonic expression is not obligatory for normal development. However, it is conceivable that there is a developmental interaction between certain MHC class II alleles such as the unique *I-Aβ* allele expressed by NOD mice (32) and *Qa/TL* gene products. Indeed, such interactions could conceivably underlie heretofore unexplained phenomena such as the reported transmission bias of "diabetogenic" HLA-DR-associated alleles to offspring of diabetic sires (33).

Regardless of the role, if any, for the NOD occult locus in diabetes pathogenesis, it represents a valuable tool for analyzing how *cis*- and *trans*-acting genomic factors combine to regulate an IFN-γ–responsive gene in an epithelial cell. Both *cis*- and *trans*-acting regulatory elements have been defined in IFN-γ induction of murine class I and II MHC gene transcription, with multiple *cis*-acting elements usually associated with consensus sequences located 5′ to the class I and II structural genes (34). *Cis*-acting element(s) apparently were responsible for the ability of NOD/Lt, but not CBA/J, islet or Mϕ to maintain significant constitutive levels of H-2K antigen in culture in the absence of IFN-γ (Table I). Important nucleotide sequence differences have been demonstrated in the upstream IFN-γ consensus sequences of human HLA-A3 and B7 class I genes (35), such that the inbred strain difference in ability to maintain class I gene expression in culture may well indicate structural differences in regulatory protein binding sites on the DNA transcriptional control elements (X and Y boxes). That the strain differences in levels of constitutive and IFN-γ–inducible class I surface antigen were not due to qualitative differences in nuclear regulatory proteins was suggested by the data from F_1 cell cultures. The data showed that the markedly different patterns of constitutive and IFN-γ–inducible class I antigen expression characteristic of each parental strain were co-expressed in F_1 cell cultures (Table I). Green et al. (36) have reported that failure of IFN-γ to induce K^k in a mouse tumor cell line was likely due to an alteration *cis* to the K^k gene rather than to the presence of *trans*-acting negative suppressors of transcription.

Tissue-specific (Mϕ vs. islet cell) variation in IFN-γ–response elements of class II genes was consistent with a study indicating that transcription from these genes in most nonleukocytic cells may normally be under active suppression, possibly mediated by IFN-α/β (37–39). F_1 data in the present study suggested qualitative differences in *trans*-suppressive proteins regulating IFN-γ–induced class II and occult gene transcription (Table I). A *trans*-acting factor emanating from the CBA/J genome completely suppressed both constitutive and IFN-γ–inducible I-A antigen expression in β cells (but not Mϕ), and in addition suppressed, in a seemingly dominant fashion, IFN-γ–inducible occult antigen expression in both cultured F_1 β cells and Mϕ. Thus, control of IFN-γ regulation of class II and occult gene expression could be dissociated, with levels of expression of the occult locus conveniently marking presence or absence of CBA-derived *trans*-suppressive factors.

Fig. 3 shows that mAb 28-13-3 was not crossreacting with the standard NOD MHC class I gene products under our culture conditions since 17 d after withdrawal of IFN-γ, cultured NOD macrophages continued to express high levels of H-2K^d (detected with mAb 31-3-4), while the IFN-γ–induced occult antigen expression had decayed to zero. This demonstrates that mAb 28-13-3 is not binding to an epitope on a standard MHC class I molecule. The extended interval of between 48 and 64 h before the occult antigen appeared on the surface of IFN-γ–treated Mϕ as compared to the more rapid appearance of I-A^{NOD} (Fig. 3) suggested that an interplay of regulatory genes was necessary before the occult gene was transcribed and final product could be detected on the surface of Mϕ. IFN-γ stimulation of surface I-A and occult antigens in the long-term cultures used in the present study probably entailed a combination of increased mRNA transcription, mRNA stabilization (as evidenced by pulse-chase study), and possibly even posttranslational effects. Constitutive class II expression in mouse B lymphocytes is under the control of at least one dominant locus, *Air-1* (activator of immune response genes) on Chr 16 encoding a *trans*-acting diffusable factor (40). The CBA/J genetic background prevented activation of IFN-γ–responsive elements controlling class II expression in β cells. The genetic basis for this remains under investigation; results of the genetic analysis for control of occult gene expression in Mϕ suggest that at least one co-dominant and non-MHC-linked autosomal gene inherited from CBA/J is involved.

In conclusion, this study provides a basis for understanding the wide variations reported in the literature in IFN-γ inducibility of class II MHC antigens on murine β cells. Inducibility is not an intrinsic property of all mouse β cells, but instead depends upon strain background-determined presence or absence of response modifying genes. The observation that IFN-γ elicits expression of a locus for a class I–like gene that may serve as a recognition element for an immune surveillance cell is of interest in view of the demonstration of a diabetogenic gene on Chr 17 (*Idd-1^s*) whose action is recessive (41). As mentioned previously, the *I-A* gene product of NOD is unique and may contribute to diabetogenesis (32). In addition, lack of expression of an *I-E* gene product has also been reported to control insulitis in NOD mice (42). The IFN-γ–inducible occult locus adds yet another candidate locus on Chr 17 whose expression may influence diabetogenesis.

Summary

This study provides a basis for understanding the wide variations reported in the literature in IFN-γ inducibility of class II MHC antigens on murine β cells. Inducibility is not an intrinsic property of all mouse β cells, but instead depends upon strain- (and tissue-) specific response modifying factors. This was demonstrated by comparison of constitutive and IFN-γ–induced class I and class II MHC gene products on cultured islet cell monolayers. Islet cultures were established from autoimmune diabetes-prone NOD/Lt mice, diabetes-resistant NON/Lt and CBA/J mice, as well as F_1 hybrids between these latter two strains and NOD/Lt. Cultures of peritoneal macrophages (Mϕ) from each strain were established as controls. After 3 wk of culture (with incubation in the presence or absence of IFN-γ during the last 6 d), constitutive expression as well as IFN-γ induction of class I MHC antigen expression was demonstrated on NOD/Lt and NON/Lt islet cells by antibody plus complement–mediated cytotoxicity. Although CBA/J islets and Mϕ did not main-

tain constitutive class I or class II antigen expression in culture in the absence of IFN-γ, class I H-2K^k antigen was IFN-γ inducible. Whereas IFN-γ-induced class II I-A^k antigen on CBA/J Mϕ, it failed to induce this antigen on CBA/J islets. In contrast, I-A antigens were IFN-γ inducible on NOD/Lt and NON/Lt islets and Mϕ. In (CBA × NOD)F$_1$ hybrids, loss of IFN-γ inducibility of the I-A^{NOD} product established that suppression was mediated by a *trans*-acting factor from the CBA/J genome.

In the course of these studies, IFN-γ inducibility of a crossreactive occult class I-like antigen on both NOD/Lt islet cell and Mϕ cultures was unexpectedly detected when mAb 28-13-3 (public specificity 39, reactive with H-2K^{b,f}) was used as a negative control. Although not detectable by cytofluorographic analysis of freshly isolated NOD/Lt splenic leukocytes, occult antigen could be induced on NOD/Lt peritoneal macrophages (Mϕ) cultured for 3 d in IFN-γ. Time course of induction showed the occult antigen to be distinct from NOD/Lt class I and II gene products. In both islet cell and Mϕ cultures established from (CBA × NOD)F$_1$ hybrids, *trans*-suppressive factor(s) from the CBA/J genome not only suppressed IFN-γ-induced expression of I-A^{NOD}, but additionally suppressed occult antigen induction. Backcross of F$_1$ to both parental strains indicated that the occult locus was on Chr 17, tightly linked to MHC. Polygenic control of IFN-γ responsiveness was indicated, and the possibility that the inducible antigen may be encoded by a *Qa*-like gene was discussed.

Diabetogenesis in NOD mice entails a T lymphocyte-mediated destruction of pancreatic β cells. Although insulitis is an histopathologic feature of diabetogenesis detected shortly after weaning, erosion of pancreatic insulin content is not observed until several months thereafter. Insulin content does not decline gradually, but appears to drop precipitously. Lymphokines such as IFN-γ from infiltrating cells could induce β cell expression of surface proteins, including MHC antigens, that may in turn activate cytotoxic effector cells. We have demonstrated the potential for such an interaction in vitro. NOD/Lt T cells primed in vivo to splenocytes from a NOD.NON-*H-2K^b* congenic stock proliferated in vitro in response to IFN-γ-treated NOD islets but not untreated NOD islets. This model system suggests the interesting possibility that accumulation of IFN-γ around NOD β cells in situ could trigger activation of NOD T cells via induction of occult antigen on β cell surfaces.

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