

Independent Genetic Regulation of T-Cell and Antigen-Presenting Cell Participation in Autoimmune Islet Inflammation

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Genetic susceptibility to type 1 diabetes in the nonobese diabetic (NOD) mouse involves at least 17 *Idd* loci. *Idd1* has been mapped to a class II gene in the major histocompatibility complex (MHC), whereas the products and functions of the remaining *Idd* loci are unresolved. To investigate how non-MHC *Idd* genes regulate islet inflammation and IDDM progression, NOD mice were compared with the nonobese diabetes-resistant (NOR) mouse, a related MHC-identical strain that possesses a subset of the NOD-derived alleles at the *Idd* loci. Using quantitative reverse transcriptase-polymerase chain reaction amplification and immunohistochemistry, we observed that disease resistance in NOR mice is reflected by a protracted block at the earliest stage of insulinitis. In NOD islets, early antigen-presenting cell (APC) recruitment to islet lesions was temporally coincident with progressive T-cell infiltration. In striking contrast, islet infiltrates in NOR mice were composed of APCs with minimal contribution from T-cells and T-cell-derived inflammatory cytokines, conferring apparent resistance to invasive insulinitis and β -cell destruction. This is the first evidence that a subset of *Idd* susceptibility loci independently regulate T-cell and APC participation in insulinitis progression. As progress is made toward identification of the *Idd* gene products, it will be crucial to determine how they regulate diabetogenesis. Our data define distinct cellular stages of IDDM pathogenesis in which the impact of *Idd* genes can be readily analyzed. *Diabetes* 47:331–338, 1998

IDDM is an autoimmune disease that affects 1 in 300–600 North Americans and has an enormous impact on health care (1). IDDM results from the progressive infiltration of the pancreatic islets of Langerhans by CD4⁺ T-helper (Th) cells, CD8⁺ T-cells, and antigen-

presenting cells (APCs) that precedes the destruction of insulin-producing β -cells (2,3). In the nonobese diabetic (NOD) mouse, T-cells and APCs are first observed at the islet periphery (peri-insulinitis) at 1 month of age (4). The frequency of affected islets and the degree of T-cell and APC infiltration increase dramatically over the next 2 months (5–9). By 6 months of age, 70–90% of female and 10–20% of male NOD animals become overtly diabetic (10). Previously, we observed that dramatic sex-based variation in proinflammatory Th1 (interleukin [IL]-2, γ -interferon [IFN- γ], and tumor necrosis factor- α [TNF- α]) and immunoprotective Th2 (IL-4) cytokine expression is evident at the first stage (stage 1) of NOD islet inflammation and predicts the severity of insulinitis at subsequent disease stages (11). These data suggest that cytokine variation imprinted during stage 1 islet inflammation influences the cellular mechanisms of IDDM onset. However, the genetic factors that regulate both the onset and progression of early insulinitis in IDDM-susceptible mice and humans are not defined.

IDDM susceptibility is linked to 17 insulin-dependent diabetes (*Idd*) loci in NOD mice (12,13), including *Idd15–17* (14–16) and is similarly complex in humans (17,18). Analyses of both human patients (19) and NOD mice (20–23) have mapped *Idd1* to the major histocompatibility complex (MHC) and have identified this locus as the primary predictor of diabetes susceptibility. The remaining 16 non-MHC *Idd* loci are important contributors because NOD.H2^b MHC-congenic mice display insulinitis but are diabetes resistant (23), whereas C57/B10.H2^{NOD} mice are both insulinitis and diabetes free (24,25). Although the identities of the remaining 16 *Idd* loci in NOD are unresolved (12), several appear to enhance disease resistance in non-diabetes-prone NOD-related strains rather than increasing IDDM susceptibility (12). An analysis of disease progression in these mice may identify subsets of *Idd* loci that regulate cellular mechanisms of IDDM pathogenesis.

The nonobese diabetes-resistant (NOR) mouse is an NOD-related MHC-syngenic recombinant strain that possesses ~12% C57/KsJ (BKs)-derived genes (26), resulting in a unique combination of resistance and susceptibility alleles at the *Idd* loci. NOR mice possess NOD-derived *Idd1*, 2, 3, 6, 7, 8, 10, 12, and 14 alleles (27,28), but the remaining 5–8 *Idd* alleles are BKs-derived (NOR mice have not been typed for *Idd15–17*). NOR mice are resistant to both spontaneous and cyclophosphamide-induced IDDM and display only peri-insulinitis up to 9 months of age (27,29). Diabetes resistance in NOR mice is recessive because the severe insulinitis characteristic of parental NOD is observed in (NOD \times NOR)-F₁

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Received for publication 26 August 1997 and accepted in revised form 12 November 1997.

ANOVA, analysis of variance; APC, antigen-presenting cell; BKs, C57/KsJ; F₁, (NOD \times NOR)-F₁; H + E, 0.1% Mayer's hematoxylin and 0.5% Eosin Y; MHC, major histocompatibility complex; IFN- γ , γ -interferon; Ig_M, immunoglobulin M constant region; IL, interleukin; NOD, nonobese diabetic; NOR, nonobese diabetes resistant; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; RT, reverse transcriptase; TCR, T-cell receptor; Th, T-helper; TNF- α , tumor necrosis factor- α .

(F₁) progeny, and 33% of F₁ animals become diabetic by 1 year of age (26,27). Thus, analysis of disease progression in NOR mice may link the cellular mechanisms that drive the onset and progression of stage 1 islet inflammation with the *Idd*-regulated pathways that differ between NOR and NOD mice.

To address the immunological basis of IDDM susceptibility and resistance, we have examined T-cell and APC participation in the progression of islet inflammation in NOR, NOD, and F₁ mice. In NOR mice, we observed a profound block at stage 1 insulinitis compared with either NOD or F₁ animals. From 1 to 6 months of age, APC accumulation in NOR islets paralleled the progressive insulinitis characteristic of NOD and F₁ islets. In NOD islet lesions, early APC recruitment was temporally coincident with progressive T-cell infiltration. Remarkably, over this same interval, APC infiltration of NOR islets occurred with minimal contribution of T-cells and T-cell-derived inflammatory cytokines, revealing an uncoupling of T-cell and APC contribution to islet inflammation. Our data identify a sequence of cellular events that drive the induction and progression of islet inflammation in IDDM-susceptible animals. Importantly, this suggests that the subset of non-MHC *Idd* genes that differ between the NOR and NOD genomes may independently regulate the kinetics of T-cell and APC participation in islet inflammation.

RESEARCH DESIGN AND METHODS

Mice. All mice used in these studies were bred and maintained at the Hospital for Sick Children, Toronto, Ontario, Canada. The incidence of spontaneous diabetes in NOD animals was 83% in females and 9% in males by 6 months of age (10). None of the NOR or F₁ animals were spontaneously diabetic at 6 months, as has been previously described (27).

Assessment of insulinitis severity as a function of age. Pancreases were removed from 4 NOD, NOR, and F₁ female littermates at 40, 80, 120, and 180 days of age and from NOR females at 270 days, embedded in Tissue-Tek (Bayer, Etobicoke, Ontario, Canada), snap-frozen in liquid nitrogen, and stored at -80°C. Before sectioning, glass slides were pretreated by a 5-min exposure to 2% 3-aminopropyltriethoxysilane (Sigma, St. Louis, MO) in acetone, rinsed in water followed by acetone, and then air-dried. Two serial 8- μ m frozen sections were cut in each block from six areas separated by 150 μ m (Leica CM 3050 cryostat, Leica Canada, Willowdale, Ontario, Canada), fixed in 100% ethanol for 10 min, and stained with 0.1% Mayer's hematoxylin and 0.5% Eosin Y (H + E) (Sigma). Insulinitis severity was assessed by scoring islets using standard methods (20) as follows. Each islet was assigned a numerical value to describe insulinitis severity: 0, no infiltrates were visible; 1, peri-insulinitis, indicated by perivascular and peri-islet infiltrates; 2, <50 intraislet infiltrates visible; 3, invasive insulinitis where <50% of the islet is occluded by leukocytes; and 4, complete infiltration of 50–100% of the islet. For each section, scores were summed and divided by the total number of islets counted to obtain an average score of insulinitis severity at each age point. Statistical analysis was performed using analysis of variance (ANOVA) software (Abacus Concepts, Berkeley, CA).

Quantitative reverse transcriptase-polymerase chain reaction. Multiple islet samples were pooled from two or three NOD, NOR, and F₁ male and female littermates of 40, 80, and 120 days of age and from female NOD and NOR littermates at 180 days of age. RNA was extracted and cDNA prepared as previously described (11). An aliquot of each islet cDNA sample, corresponding to the RNA equivalence of one islet, was analyzed by quantitative polymerase chain reaction (PCR) for the expression of the APC-specific gene products MHC class II I- β , B7-1, B7-2, and immunoglobulin M constant region (I μ), and the T-cell-specific gene products CD3 ϵ , IL-2, IL-4, IFN- γ , and TNF- α , as previously described (11,30). Briefly, islet cDNA samples were coincidentally PCR amplified with a titration of plasmid standard templates that contained the PCR amplification product of each gene of interest. The resultant PCR products were electrophoresed through agarose gels, Southern blotted, and digitized by a phosphorimaging scanner (Molecular Dynamics, Sunnyvale, CA). After densitometry using ImageQuant software (Molecular Dynamics), the plasmid standard titration enabled the generation of a standard curve that compared input template number to output signal intensity. The slope of the linear portion of this curve was then used to interpolate template number from quantified signal output in islet cDNA samples. Correction for the variation in cDNA amount between different islet samples was made

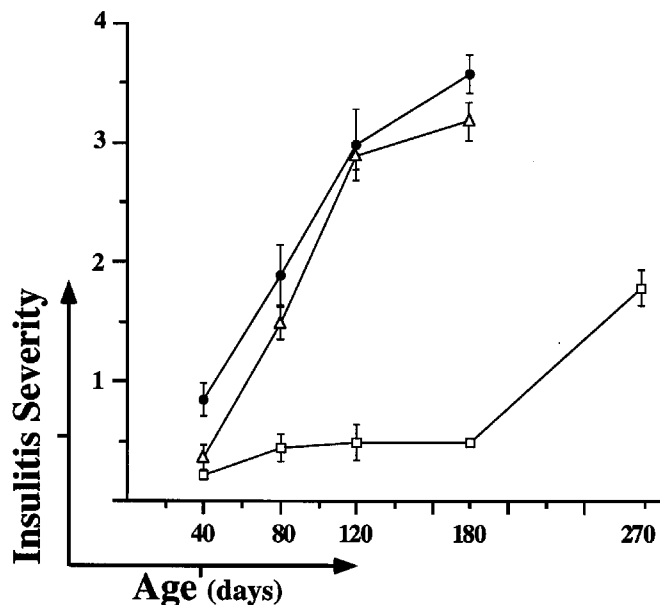


FIG. 1. Insulinitis severity did not increase as a function of age in NOR mice. Islets in H + E-stained sections of pancreases from female NOD, F₁, and NOR mice at 40, 80, 120, and 180 days of age and from NOR females at 270 days were scored for insulinitis severity (Table 1). NOR (□), NOD (●), and F₁ (△). For each age point, islet scores were summed and divided by the total number of islets, to obtain an average score of insulinitis severity. Average insulinitis score \pm SE is shown.

using PCR amplification of β -actin. Full experimental details of these procedures have been recently published (11,30).

Immunoperoxidase staining. Frozen pancreatic sections were prepared from NOD and NOR female mice at 40, 80, and 120 days of age as described above, and immunoperoxidase staining was performed as follows. Serial sections were incubated for 20 min in 1% hydrogen peroxide to destroy endogenous peroxidase activity, rinsed in phosphate-buffered saline (PBS), and then incubated for 60 min with biotin-conjugated anti-T-cell receptor (TCR) $\alpha\beta$ (H57-597) (31) or anti-I- β ⁸⁷ (10-2.16) (32) antibodies in PBS plus 0.1% bovine serum albumin (Sigma). Antibodies were purified from hybridoma culture supernatants and biotin-conjugated using standard methods. Sections were then incubated for 30 min in streptavidin peroxidase (Sigma), rinsed, and exposed to diaminobenzidine tetrahydrochloride (Sigma) according to the manufacturer's instructions. Dark coloration indicated positive staining. Sections were counterstained in H + E. Micrographs were taken with a DMLD Microscope (Leica).

RESULTS

Islet inflammation in NOR mice was blocked at peri-insulinitis. Previous studies of NOR animals have observed peri-insulinitis (histological severity score of 1) in 9-month-old mice (younger animals were not examined) (27). Originally, resistance to invasive insulinitis was also reported in 9-month-old F₁ progeny (27), but in a subsequent report, severe insulinitis in younger F₁ animals, characteristic of NOD parents, was observed (26). To clarify the kinetics of insulinitis in NOD, NOR, and F₁ animals, we scored insulinitis severity in H + E-stained pancreatic sections from female mice at 40, 80, 120, and 180 days of age and from NOR mice at 270 days. In agreement with previous observations (26), the age-linked progression of islet inflammation in F₁ and parental NOD pancreases (Fig. 1, Table 1) was similar ($P = 0.6548$, one-factor ANOVA). In contrast, peri-insulinitis predominated in NOR mice between 40 and 180 days of age. For example, insulinitis severity in NOD increased from an average score of 0.85 ± 0.14

TABLE 1
Insulinitis severity in NOD and NOR islets

Age (days)	Strain	Number islets scored	Insulinitis severity score				
			0	1	2	3	4
40	NOD	344	66 ± 7.5	11 ± 5.3	7.8 ± 2.3	8.5 ± 3.3	8.0 ± 3.5
	F ₁	270	72 ± 6.2	14 ± 1.4	8.0 ± 3.6	4.0 ± 0.9	0
	NOR	219	79 ± 1.5	21 ± 3.4	<0.5	0	0
80	NOD	286	30 ± 4.3	21 ± 5.8	12 ± 3.1	12 ± 1.0	26 ± 8.5
	F ₁	225	45 ± 3.7	15 ± 2.3	8.2 ± 2.4	15 ± 1.5	17 ± 2.9
	NOR	196	63 ± 6.6	31 ± 0.9	7.0 ± 3.5	<0.5	<0.5
120	NOD	220	6.8 ± 1.2	8.7 ± 2.3	9.0 ± 1.0	23 ± 6.9	53 ± 11
	F ₁	212	6.0 ± 2.4	11 ± 2.6	12 ± 6.6	20 ± 4.6	42 ± 20
	NOR	278	63 ± 6.2	26 ± 5.8	11 ± 3.5	1.3 ± 0.9	0
180	NOD	136	<0.5	<0.5	9.5 ± 1.0	12 ± 4.5	78 ± 36
	F ₁	150	4.0 ± 1.0	7.3 ± 1.2	13 ± 2.2	22 ± 5.7	55 ± 12
	NOR	282	60 ± 4.4	25 ± 4.3	11 ± 3.3	1.9 ± 0.5	1.3 ± 0.9
270	NOR	215	33 ± 2.5	18 ± 1.4	19 ± 7.1	16 ± 8.3	18 ± 9.5

Data are means ± SE. Invasive insulinitis was significantly reduced in NOR islets before 270 days of age. Each islet was assigned a numerical value to describe insulinitis severity: 0 (no infiltration), 1 (peri-insulinitis), 2 (<50 intra-islet infiltrates visible), 3 (invasive insulinitis with <50% of the islet occluded by leukocytes), and 4 (complete infiltration of 50–100% of the islet). For each age point, the percentage of islets scored in each stage is expressed as a function of the total number of islets examined. The average insulinitis score is shown in Fig. 1.

at 40 days of age to 3.6 ± 0.01 at 180 days, whereas in NOR pancreases, no increase was observed over this interval (0.45 ± 0.11 and 0.50 ± 0.02 , respectively, $P = 0.0001$). As outlined in Table 1, invasive insulinitis (severity score >2) was rarely observed in NOR pancreases before 180 days, whereas >70% of NOD islets at 120 days were scored >2. Remarkably, the severity of islet infiltration in 270-day-old NOR mice still did not exceed that observed in 80-day-old NOD animals (1.8 ± 0.07 vs. 1.9 ± 0.25 , respectively). Thus, disease resistance in NOR mice was reflected by a protracted delay or block at stage 1 of insulinitis progression that extended from 1 to 9 months of age.

The kinetics of APC infiltration in NOR islets were dissociated from T-cells. Although invasive insulinitis was rare, the frequency of infiltrated NOR islets doubled between 40 and 80 or 120 days (Table 1). To examine T-cell and APC-specific markers of immunological effector function, we used a sensitive, quantitative reverse transcriptase (RT)-PCR analysis of insulinitis progression. Islet RNA was prepared from NOD, NOR, and F₁ mice 40, 80, 120, and 180 days of age. As we recently described (11), this experimental design enables quantitation of low levels of leukocyte-specific transcripts in a large excess of islet cell RNA. We analyzed the expression of I-A β^{g7} as an APC marker, Ig μ as a B-cell marker, and CD3 ϵ to indicate T-cell infiltration (Fig. 2). We have shown previously that CD3 ϵ transcript levels accurately reflect T-cell number in NOD islet lesions and that expression of this gene does not vary as a function of T-cell activation (11). Expression of these genes was identical in male and female NOR islets at all ages examined (data not shown). At 40 days of age, I-A β^{g7} expression in female NOR islets was 60-fold lower than age-matched NOD or F₁ islets (Fig. 2A). From 40 to 80 days, I-A β^{g7} transcripts increased 50-fold in NOR, NOD, and F₁ islets, and a further two- to fivefold by 180 days. Ig μ expression also increased between 40 and 80 days in NOR islet lesions (Fig. 2B), but remained 10- to 150-fold lower than age-matched NOD or F₁ islets at later time points.

Although I-A β^{g7} , Ig μ , and CD3 ϵ expression rose coincidentally in NOD and F₁ islets, accumulation of APC-specific transcripts in NOR islets occurred without an increase in CD3 ϵ expression between 40 and 80 days of age (Fig. 2C). Further, CD3 ϵ transcript levels in these NOR lesions were 150-fold lower at 40 days, and 4,000-fold lower at 80 days ($P = 0.0001$) compared with age-matched NOD or F₁ animals. Although CD3 ϵ expression increased after 80 days, transcript levels in 180-day-old NOR islets were lower than those in 40-day-old NOD islets at the onset of islet inflammation. These data demonstrate that the expression of APC-specific genes increased as a function of age in NOR, NOD, and F₁ islets. In contrast, while T-cell and APC recruitment occurs concomitantly in diabetes-prone NOD and F₁ islets, T-cell accumulation in NOR lesions was delayed. These results demonstrate that the kinetics of T-cell and APC recruitment can be dissociated during the induction and early progression of stage 1 islet inflammation.

The cytokines in NOR islets. We previously reported that IL-2 and IL-4 mRNA and protein expression peaked within 24 h of T-cell activation induced TCR ligation, suggesting that Th cytokine transcripts present in islet lesions indicate recently activated T-cells (11). We also found that the expression of the proinflammatory Th1 cytokines IL-2, IFN- γ , and TNF- α during stage 1 insulinitis in NOD females predicted subsequent β -cell destruction, whereas increased expression of the Th2 cytokine IL-4 at stage 1 was protective in NOD males (11). To determine if the strain-dependent variation in CD3 ϵ expression was associated with distinct Th cytokine profiles of islet-infiltrating T-cells, we analyzed expression of IL-2, IFN- γ , TNF- α , and IL-4 in NOR, NOD, and F₁ islets. Each of these cytokines was detectable in NOR islets at 120 and 180 days of age (Fig. 3), consistent with the increased CD3 ϵ transcript levels detected at these age points. However, neither IL-2 (Fig. 3A) nor TNF- α (Fig. 3B) was detected above background (<10² templates) in NOR islets before 120 days, in contrast to high levels of these cytokine transcripts in age-

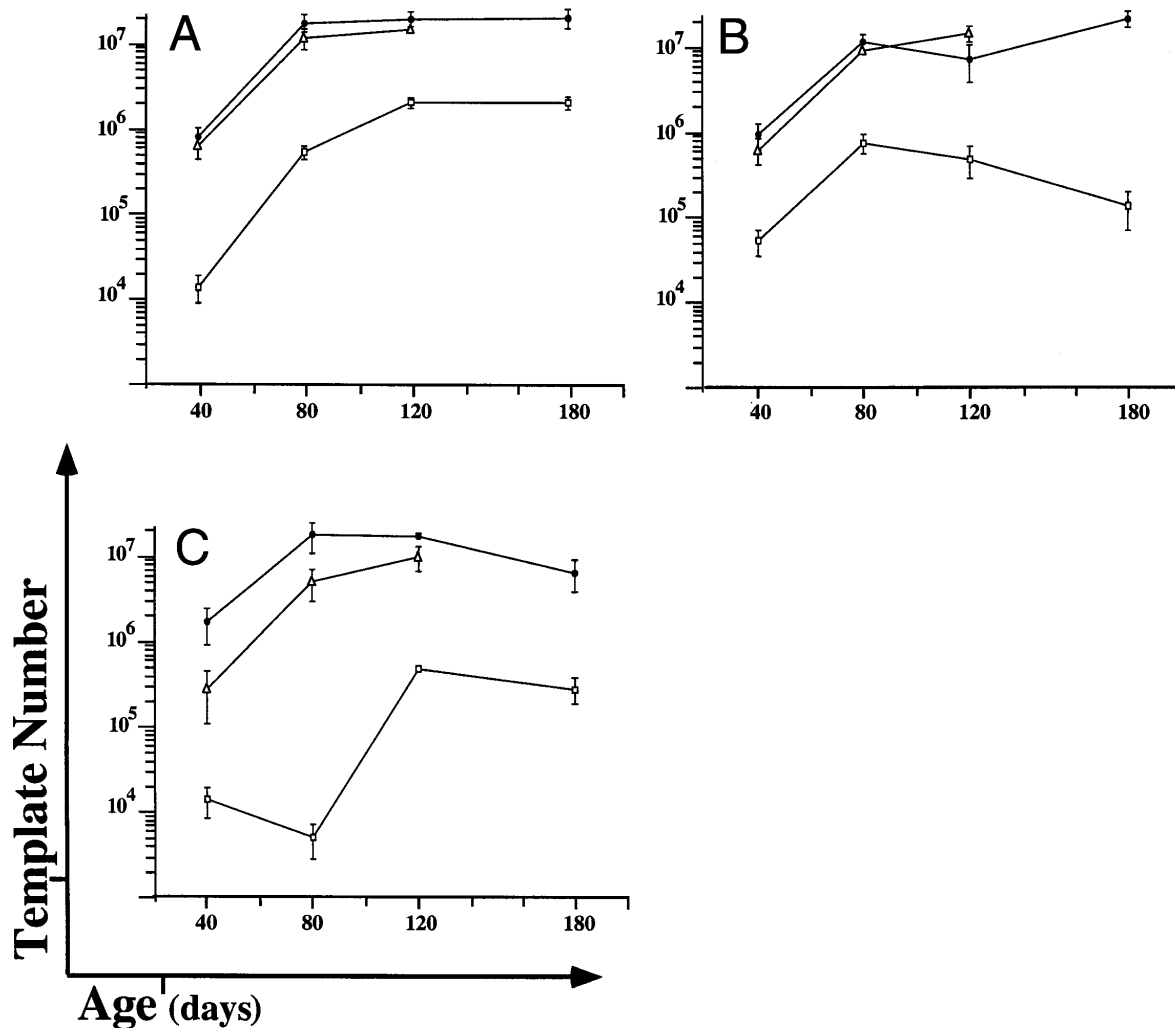


FIG. 2. I-A β^{g7} and Ig, but not CD3 ϵ , transcript levels increase between 40 and 80 days in NOR, NOD, and F₁ islets. Islet cDNA samples prepared from NOR (□), NOD (●), and F₁ (Δ) females 40, 80, and 120 days of age and from NOD and NOR females at 180 days were PCR amplified for the following: A: MHC class II I-A β ; B: Ig; C: CD3 ϵ . Expression in nondiabetes susceptible strains is $<10^2$ templates for each gene of interest (11).

matched NOD and F₁ islets. IFN- γ (Fig. 3C) expression in NOR islets at 40 days was 400-fold lower than in age-matched NOD. Interestingly, IL-4 was the only cytokine detected in NOR islets at all age points, although at levels 50-fold lower than in age-matched NOD (Fig. 3D). These data demonstrate that, before 120 days of age, recruitment of activated proinflammatory Th1-cells to NOR islets was extremely limited compared with NOD lesions.

These data indicated that diabetes resistance in NOR mice correlated with a profound delay in the kinetics of Th1-cell, but not APC, accumulation in islet lesions. To examine the presence and distribution of T-cells and APCs during insulinitis progression, serial frozen sections of NOD and NOR pancreases were stained with antibodies to TCR $\alpha\beta$ and I-A β^{g7} . Representative micrographs are shown (Fig. 4). Coincident expression of I-A β^{g7} and TCR $\alpha\beta$ was detected in NOD islets at 40, 80, and 120 days of age (Fig. 4). In contrast, I-A β^{g7} protein was evident, but TCR $\alpha\beta$ protein was virtually undetectable, in 40-day-old NOR islets (Fig. 4H and N). At 80 days, coincident TCR $\alpha\beta$ and I-A β^{g7} protein expression was rarely found in NOR islets (Fig. 4J and P). By 120 days, TCR $\alpha\beta^+$ cells

were observed in NOR islets (Fig. 4R), consistent with the increased CD3 ϵ expression detected by RT-PCR at this age (Fig. 2C). These data confirm that MHC class II⁺ APCs were present in NOR islet lesions containing minimal TCR β^+ T-cells. Thus, the block at peri-insulinitis in NOR correlates with the dissociation of T-cell and APC participation during the first stage of disease induction.

DISCUSSION

We report a comparative analysis of insulinitis in the NOD mouse and the NOR strain that reveals independent regulation of APC and T-cell participation in the development of islet inflammation. Whereas insulinitis onset was temporally coincident in NOD and NOR animals, the composition of infiltrating cells, and subsequent disease progression, was strikingly different between the two strains. In NOD mice, both APCs and T-cells were observed in stage 1 infiltrates, and subsequent expression of proinflammatory Th1 cytokines correlated with rapid and severe infiltration of the islet interior. In NOR mice, however, stage 1 infiltrates contained APCs with minimal T-cell content, and without the detectable proin-

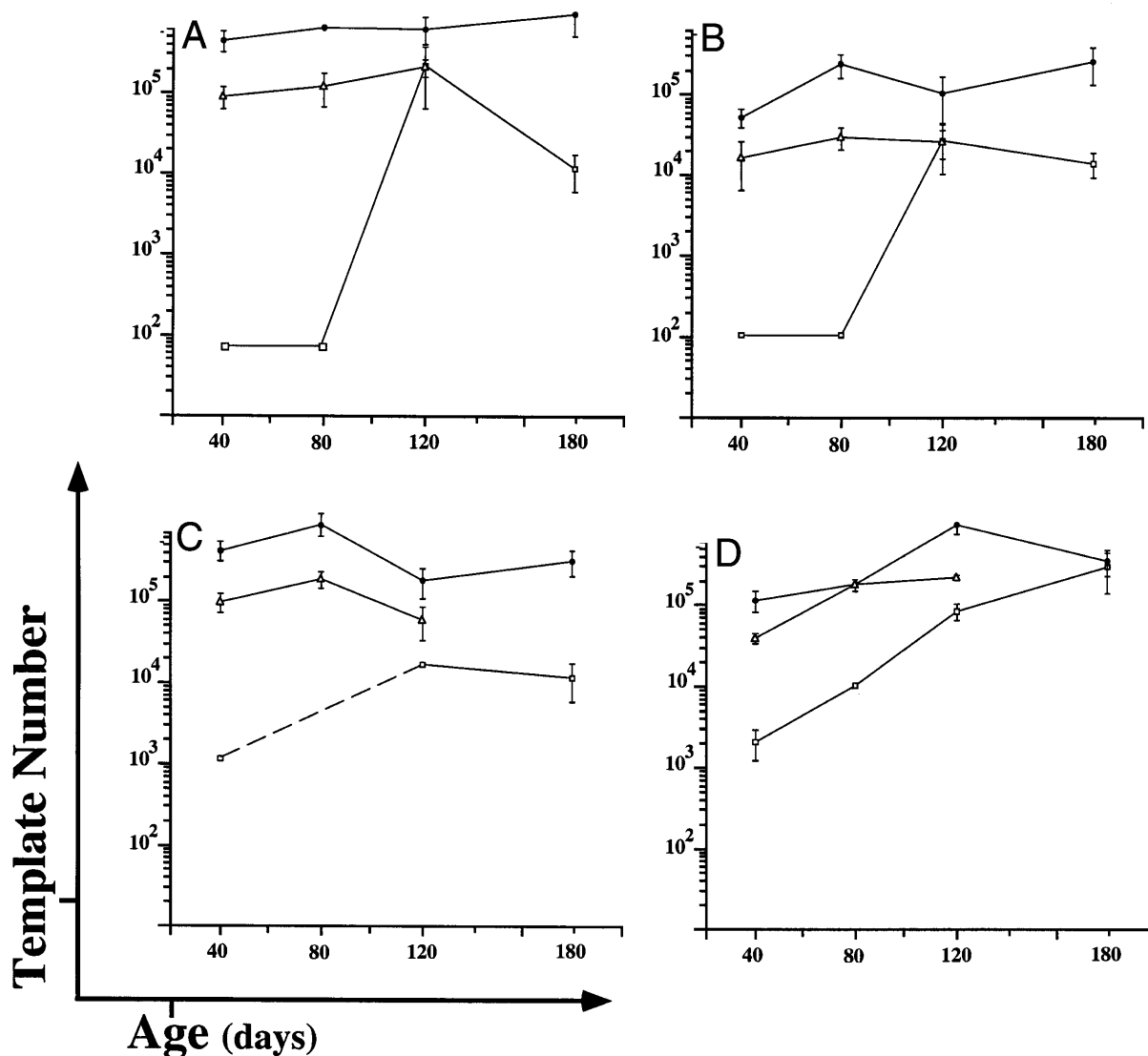


FIG. 3. Th cytokine transcripts are reduced in NOR islets compared with age-matched NOD. Islet cDNA samples prepared from NOR (□), NOD (●), and F₁ (△) females were PCR amplified for the following: A: IL-2; B: TNF- α ; C: IFN- γ ; D: IL-4 (the dashed line indicates IFN- γ transcripts detected in NOR islets at 40, but not 80, days of age). Expression in nondiabetic susceptible strains is $<10^2$ templates for each gene of interest (11).

inflammatory cytokines IL-2 and TNF- α . The paucity of Th1 effector cells in NOR islets correlated with an arrest at the peri-insulinitis stage. The NOR-resistance phenotype was recessive because disease progression in F₁ progeny was indistinguishable from NOD parents. These observations identify a sequence of cellular events that drive the induction and progression of islet inflammation in IDDM-susceptible animals. Importantly, we suggest that the subset of non-MHC *Idd* genes that differ between the NOR and NOD genome independently regulate the kinetics of T-cell and APC participation in islet inflammation.

APCs drive peri-insulinitis onset. In NOR mice, peri-insulinitis was characterized by a progressive increase in the expression of APC-specific genes despite extremely low levels of CD3 ϵ expression, reflecting nominal T-cell involvement. These results suggest that APCs drive peri-insulinitis onset and that this early event is not dependent on coincident T-cell migration. Several studies have implicated APCs in the gen-

esis and early progression of peri-insulinitis in NOD mice. For example, spontaneous insulinitis onset in 20-day-old NOD animals is primarily mediated by the APC influx that precedes T-cell accumulation in the islets (11). Strikingly, peri-insulinitis is completely absent in NOD mice depleted of macrophages by silica treatment between 4 and 10 weeks of age (33), whereas silica treatment has no effect after the onset of invasive insulinitis in 3- to 5-month-old females (34,35). Similarly, adoptively transferred diabetes is arrested at the peri-insulinitis stage in NOD recipients pretreated with an antibody that disables macrophage migration (36). B-cells are also crucial to early disease progression because peri-insulinitis is absent in young NOD animals depleted of B-cells by anti- μ antibody treatment (38) or in NOD mice carrying a targeted disruption of the Ig μ locus that prevents B-cell maturation (39). Our study argues that the critical role of professional APCs, including B-cells, is in the induction phase of peri-insulinitis. Importantly, we also show that peri-insulinitis onset can be

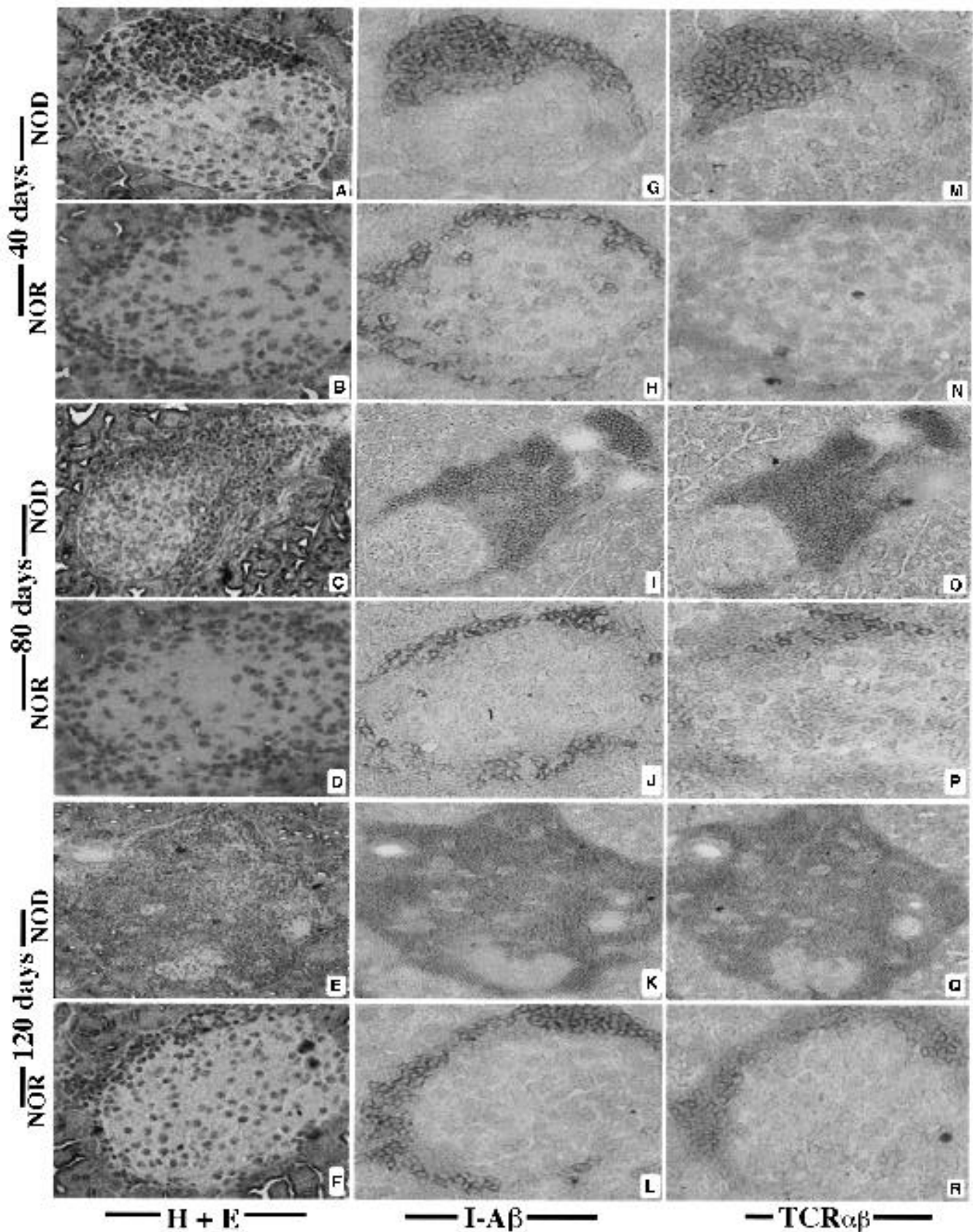


FIG. 4. Coincident TCR $\alpha\beta$ ⁺ and I-A β ⁸⁷ protein staining was reduced in NOR islets before 120 days of age. Islets from serial sections of 40-, 80-, and 120-day-old NOR and NOD female mice are shown stained with H + E to visualize infiltrating leukocytes (A-F), and I-A β ⁸⁷ (G-L) and TCR $\alpha\beta$ (M-R) protein expression using 10.2.16 and H57-597 antibodies, respectively. Positive protein expression by islet infiltrating cells is indicated by dark coloration. Original magnification $\times 250$.

dissociated from the progression to invasive insulinitis and implicate a subset of non-MHC *Idd* genes in the regulation of these events.

Progression to invasive insulinitis is T-cell dependent. In NOR mice, peri-insulinitis (stage 1) was protracted and characterized by limited migration of Th1 effector cells to, or their retention in, islet lesions. Significantly, these data suggest that the progression from stage 1 to invasive insulinitis (stage 2) and β -cell destruction (stage 3) requires the participation of Th1 effector cells in islet lesions (11). Independent support for this idea comes from previous studies showing that a protracted interval of peri-insulinitis in NOD mice is induced after T-cell depletion by anti-CD3 (40,41), anti-CD8 (35), or anti-TCR (42) antibodies. Reciprocally, rapid insulinitis and severe β -cell destruction can be provoked in non-diabetes susceptible mouse strains by islet β -cell-specific expression of proinflammatory Th1 cytokine transgenes, including IL-2, IFN- γ , or TNF- α (43). High-level expression of these cytokines in NOD islets also predicts β -cell loss in spontaneous IDDM (44,45). Previously, we showed that Th cytokine biases present during stage 1 islet inflammation have significant effects on later APC and T-cell recruitment to NOD islets (11). Expression of the immunomodulatory Th2 cytokine IL-4 in male NOD islets during stage 1 is associated with an arrest in disease progression and protection from cell loss (11). Here, we report that the virtual absence of Th1 cytokine expression in NOR islets results in a profound delay in inflammatory progression, conferring resistance to invasive insulinitis and β -cell destruction.

This study demonstrates that the temporal uncoupling of APC and T-cell participation in NOR islet inflammation dissociates stage 1 peri-insulinitis from the progression to stage 2 invasive insulinitis. Because NOR mice possess only a subset of NOD-derived *Idd* alleles, these critical events in early IDDM progression may be subject to independent genetic regulation. For example, APC infiltration and peri-insulinitis onset may be regulated by the NOD-derived *Idd* susceptibility loci (*Idd* 2, 3, 6, 7, 8, 10, 12, and 14) that are common to NOD and NOR. In addition, BKS-derived *Idd* alleles in the NOR genome may confer resistance to the selection, recruitment, and/or retention of islet-reactive T-cells, arresting disease progression at stage 1. Importantly, our observation that disease progression in F₁ progeny mimics parental NOD animals suggests that the NOR resistance phenotype is recessive. These results are consistent with a previous study by Serreze et al. (26) that identifies the BKS-derived *Idd13* as a resistance allele in NOR animals.

Previously, *Idd* loci were identified in F₁ or F₂ NOD-backcross progeny because of altered diabetes incidence (12,13). Detailed mapping of these loci has been complicated by combinatorial interactions between several *Idd* genes, which can result in incomplete penetrance of individual alleles (13). For example, the combination of *Idd3* and *Idd10* alleles derived from a nondiabetic strain confer diabetes resistance, but neither *Idd3* nor *Idd10* affects diabetes frequency alone (46). In addition, some loci, such as NOD-derived *Idd5*, which is absent in NOR, influence the timing of insulinitis onset rather than diabetes frequency (47). As investigators progress toward identification of the many *Idd* gene products, the crucial question will become the manner in which they impact diabetes progression. Our data illustrate distinct cellular stages of IDDM pathogenesis, thus resolving disease

sequelae in which the impact of *Idd* genes can be readily analyzed. A future goal will be to dissect the contributions of the *Idd* loci and understand how they control cellular mechanisms of autoreactive T-cell selection, islet inflammation, and β -cell destruction.

ACKNOWLEDGMENTS

These studies were supported by grants to J.S.D. from the Canadian Diabetes Association. C.J.F. is the recipient of a University of Toronto Open Fellowship and a Hospital for Sick Children Research Training Fellowship. J.S.D. is a Research Scientist of the National Cancer Institute of Canada.

We thank Drs. Philippe Poussier and Cynthia Guidos for their critical reviews of this manuscript.

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