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Susceptibility to diabetes is widely distributed in normal class II^u haplotype rats

K. E. Ellerman, A. A. Like

Department of Pathology, University of Massachusetts Medical School, Worcester, Mass., USA

Abstract

Aims/hypothesis. We did experiments to explore the pathways putatively leading to Type I (insulin-dependent) diabetes mellitus, and their association with the MHC locus, the major genetic determinant of disease susceptibility.
Methods. Normal MHC congenic rat strains that do not spontaneously develop diabetes or any other autoimmune syndrome were injected with the interferon-alpha inducer polyinosinic-polycytidylic acid (Poly IC).
Results. Insulitis and diabetes developed only in strains expressing Class II^u genes and was independent of the Class I haplotype. Poly IC induced islet cell Class I hyperexpression, up regulation of pancreatic endothelial intercellular adhesion molecule-1 and vascular adhesion molecule-1 and a T-cell and macrophage infiltration of the pancreatic interstitium in all rat strains studied, including diabetes-resistant strains. Poly IC also induced the generation of diabetes-transferring spleen cells in most Class II^u haplotype rats, including the diabetes-resistant *WF* rat.
Conclusion/Interpretation. The minimum requirements for autoimmune diabetes development in the rat include: *RT1* Class II^u genes, a T-cell repertoire containing beta-cell autoreactive T cells and a triggering event which breaks tolerance by the local up regulation of pancreatic endothelial adhesion receptors. Even when all of the minimum requirements have, however, been met, most Class II^u rats do not develop diabetes in response to autoimmune stimuli. It is clear, nonetheless, that susceptibility to diabetes is widely distributed in the *RT1^u* rat. [Diabetologia (2000) 43: 890–898]

Keywords Autoimmunity, *BB* rat, MHC Class II, adhesion molecules.

Type I (insulin-dependent) diabetes mellitus is a T cell-mediated disease of polygenic origin in man, mouse and rat, which is strongly associated with MHC Class II susceptibility alleles [1–3]. In the rat, spontaneous autoimmune diabetes has been reported only in the *BB/Wor* diabetes-prone (DP) [4–5] and *LETL* strains [6–7], who share the *RT1^u* MHC haplotype. Experimental infection with Kilham rat virus (KRV) will induce Type I diabetes in *BB/Wor* diabetes-resistant (DR) [8] and *LEW1.WR1* [9] rats, who share Class I *A^u* and Class II *B/D^u* alleles. The *WAG* (A. Like, unpublished observation) and *WF* [8] rats, who also bear the *RT1^u* haplotype, do not, however, develop insulitis or diabetes after infection with KRV. Further *PVG.RT1^u* rats develop an autoimmune diabetes after thymectomy and a series of γ-irradiations [10] and after treatment with KRV and Poly IC [9]. Finally, the *PVG.RT1^c* rat has been reported to develop Type I diabetes, also after thymectomy and irradiation [11]. All these studies suggest that diabetes susceptibility genes are widely distribut-

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Corresponding author: K. Ellerman, PhD., Torrey Pines Institute for Molecular Studies, 3550 General Atomics Court, San Diego, CA 92121, USA
Abbreviations: DP, diabetes-prone; DR, diabetes-resistant; IFN-α, interferon-alpha; KRV, Kilham rat virus; mAB, monoclonal antibody; Poly IC, polyinosinic-polycytidylic acid; ICAM, intercellular adhesion molecule-1; VCAM-1, vascular adhesion molecule-1

Table 1. Poly IC induces autoimmune diabetes in non-diabetes prone rat strains

Strain	RT1 A, B/D, C	Diabetes	Insulitis	Mean time (days) to diabetes
LEW1.WR1	u/u/a	22/22	22/22	5
PVG.RT1^{u}	u/u/u	26/30	25/28	5
PVG.R8	a/u/u	2/10 6/9[a]	2/10 6/9[a]	8
PVG.R23	u/a/a	0/10 0/10[a]	0/10 0/10[a]	–
PVG.RT1^{c}	c/c/c	0/12 0/5[b] 0/7[a]	0/12 0/5[b] 0/7[a]	– – –
WF	u/u/u	0/7 1/15[a]	0/7 1/15[a]	– 5[a]
LOU	u/u/u	0/6	0/6	–
WAG	u/u/u	1/9	2/8	0

Rats were injected with 7.5 μg/g body weight Poly IC, 5 days a week for 2 weeks (unless otherwise noted). The mean time to diabetes was calculated from the last day of Poly IC injections to the first day of glycosuria and hyperglycaemia (blood glucose ≥ 13.8 mmol/l). 0/9 PBS-injected *PVG.RT1*^{u}, 0/5 PBS-injected *LEW1.WR1* and 0/6 PBS-injected *PVG.R8* rats developed insulitis or diabetes. [a] 3 weeks of Poly IC injections at 7.5 μg/g body weight, [b] 2 weeks of Poly IC injections at 10 μg/g body weight

ed in the rat and susceptibility could map to regions outside the MHC. To explore the pathways which lead to autoimmunity as well as their association with the MHC, we made use of the interferon-α (IFN-α)-inducer, Poly IC, and a series of *RT1*^{u} congenic rat strains, with differing constellations of Class I and Class II genes. None of the *RT1* congenics are predisposed to spontaneous autoimmunity or have mutant *RT1* alleles.

Polyinosinic-polycytidylic acid (Poly IC) is a synthetic double-stranded RNA which induces brisk production of Type I interferons in vivo [12–13]. Poly IC has traditionally been used as an experimental surrogate for viral infections, which also induce the synthesis of Type I interferons (α and *β*) [14]. Double-stranded RNA is thought to be the interferon-inducing intermediate produced during viral infection [12]. Finally, injections of Poly IC accelerate the onset of Type I diabetes in the *BB-DP* rat [15], and induce diabetes in the coisogenic BB diabetes-resistant rat [16].

Materials and methods

Animals. Inbred *BB-DP/Wor*, *LEW1.WR1*, *PVG.RT1*^{u}, *PVG.R8*, *PVG.R23*, *WAG* and athymic *WAG/rnu* rats were raised at the University of Massachusetts Medical Center under viral antibody-free conditions. Inbred *WF*, *LOU*, and *PVG.RT1*^{c} rats were obtained from Harlan Sprague Dawley (Indianapolis, Ind., USA). All animal studies were approved by the Institutional Animal Care and Use Committee.

Treatment with Poly IC. Male and female rats, 4–5 weeks of age, were used in all experiments. Rats were injected i.p. with 7.5 μg/g body weight Poly IC (P1530, Sigma, St. Louis, Mo., USA), 5 days/week (Monday to Friday) for 2 weeks (or 3 weeks as noted in Table 1). Other than the induction of Type I diabetes in susceptible strains, no morbidity or mortality was associated with this dose of Poly IC. Poly IC was dissolved in PBS. Control rats were injected with PBS.

Detection of diabetes. After 2 weeks of Poly IC treatment, animals were tested for glycosuria three times weekly for 3 weeks. Diabetes was diagnosed on the basis of glycosuria (TesTape, Lilly, Indianapolis, Ind., USA) and a blood glucose concentration of 13.8 mmol/l or more in tail blood (Beckman Glucose Analyzer II, Beckman, Fullerton, Calif., USA). Diabetic rats were killed on the day of detection, and the non-diabetic rats were killed at the end of the 3-week monitoring period.

Morphologic studies. Pancreata and thyroids were fixed in Bouins solution and embedded in paraffin. Haematoxylin-eosin stained sections were examined for the presence of lymphocytic insulitis and thyroiditis. Tissues were removed from diabetic rats on the day of detection, and from the non-diabetic rats at the conclusion of the study.

Con A-activation of spleen cells. Spleens were harvested after 2 weeks of Poly IC or PBS injections, processed into single cell suspensions and cultured at 5×10^6 cells/ml in RPMI 1640, 2 mmol/l L-glutamine, 10 mmol/l HEPES, 5×10^{-5} mol/l 2-mercaptoethanol, 5% FCS (Hyclone, Logan Utah, USA), and 4 μg/ml Con A (ICN, Costa Mesa, Calif., USA) for 3 days at 37 °C and 6.5% CO_2. Activated cells were washed three times in RPMI before injection.

Adoptive transfer of diabetes. One spleen equivalent of Con A-activated spleen cells was injected i.p. into 21 to 25-day-old syngeneic, *BB-DP/Wor* or *WAG/rnu* recipients. Injected rats were monitored for 3 weeks for the development of glycosuria and hyperglycaemia (blood glucose ≥ 13.8 mmol/l). Diabetic rats were killed on the day of detection and the non-diabetic rats at the end of the 3-week monitoring period. All recipient rats were examined for insulitis.

Monoclonal antibodies (mABs). The mouse hybridoma cell lines OX-8 (which binds rat CD8 α-chain), OX-18 (which binds non-polymorphic RT1A Class I molecules), OX-19 (which binds all rat T cells), OX-42 (which binds rat CD11b on monocytes and macrophages), 1A29 [which binds rat intercellular adhesion molecule-1 (ICAM-1) (CD54)], and 341 (which binds rat CD8 *β*-chain expressed on $CD8^+$ T cells but not on $CD4^+$ T cells or NK cells) were used to produce tissue culture supernatants for immunostaining. Culture supernatants were used undiluted. The ED-1 antibody (Serotec, Raleigh, N.C., USA) reacts with most rat macrophages and some dendritic cells; ED-1 ascites was used at a dilution of 1:100. The rat vascular cell all adhesion molecule 1 (VCAM-1) (CD106) antibody (Babco, Berkeley, Calif., USA) was used at a dilution of 1:300. Antibodies were diluted in PBS-1% horse serum (HS).

Immunostaining. Pancreata were immersed in ornithine carbamoyltransferase (OCT) embedding medium and snap-frozen in liquid nitrogen-cooled isopentane before sectioning. Cryostat sections (5 μ) were mounted on slides, fixed in acetone at –20 °C, and stored at –20 °C. For staining, slides were immersed in acetone (10 min), air-dried, washed in PBS-1% horse serum and then immersed in 3% H_2O_2-1% BSA for 10 min. Slides were sequentially blocked with 0.5% avidin

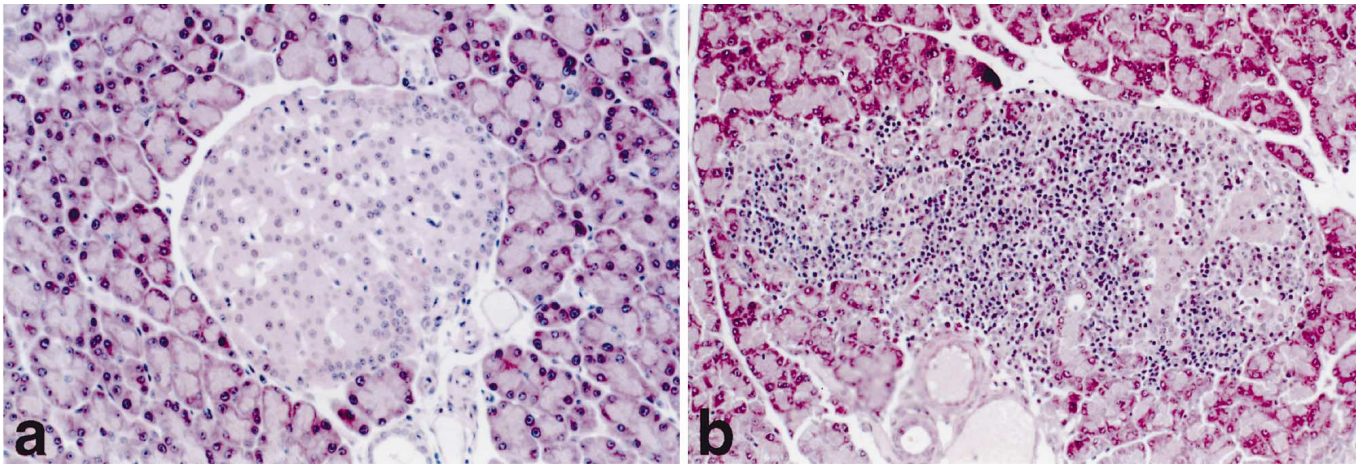

Fig. 1 A, B. Class II^u but not Class II^c rats develop insulitis and diabetes after Poly IC injections. Illustrated are pancreatic sections of Poly IC-treated rats stained with haemotoxylin and eosin. **A** The diabetes-resistant PVG^c rat was treated for 3 weeks with Poly IC and followed for another 3 weeks for the development of hyperglycaemia. There is no evidence of insulitis. **B** The Class *II^u* LEW1.WR1 rat was treated for 2 weeks with Poly IC: hyperglycaemia developed 7days after the last injection. Lymphocytic insulitis and beta-cell destruction are present

and 0.1 % biotin for 20 min to quench endogenous biotin-reactive sites. Sections were incubated with antibody for 30 min., followed by biotinylated horse anti-mouse IgG (1:100) (Vector Labs, Burlingame, Calif., USA) (30 min) and avidin-horseradish peroxidase (1:200) (Vector Labs) for 30 min. Reaction product was developed with diaminobenzidine (2 mg/ml). The counterstain was methylene blue. Staining procedures were done at room temperature.

Scoring. Specimens were scored by two independent observers. Syngeneic rats injected with PBS served as baseline controls for immunostaining. The scoring system for endothelial adhesion receptors was 0 = no staining or constitutive levels (as seen in controls), 1 + = increased staining compared with PBS controls, 2 + = moderately intense staining of many vessels, 3 + = intense staining that is widespread. Scores for T cells and macrophages were based on the degree of infiltration of positive cells; 1 + = sparse infiltration of positive cells, 2 + = moderate cellular infiltration, 3 + = extensive infiltrates of positive cells.

Results

Autoimmune diabetes is induced in many non-diabetes prone rat strains by Poly IC treatment. To explore the spectrum of diabetes susceptibility in normal rats, inbred and MHC congenic rat strains were injected with Poly IC. Injections were given 5 days a week for 2 weeks, and in some rats, 3 weeks. Controls were injected with diluent (PBS). Injected animals were then followed for 3 weeks for the development of glycosuria and hyperglycaemia (blood glucose ≥ 13.8 mmol/l). The rat MHC is denoted as RT1, where A and C are Class I loci and B/D are Class II loci.

Treatment with Poly IC induces autoimmune diabetes in normal *LEW1.WR1*, *PVG.RT1^u*, *PVG.R8*, *WF*, and *WAG* strains (Table 1). The incidence of insulitis and diabetes varied between strains: 100 % of *LEW1.WR1* rats developed diabetes after 2 weeks of Poly IC injections, whereas only 1/15 *WF* rats became diabetic after 3 weeks of Poly IC. Insulitis and diabetes appeared only in strains expressing Class II^u genes. The development of insulitis and diabetes was independent of the Class I haplotype, occurring in both *LEW1.WR1* (Class I A^{u}C^a) and *PVG.R8* (Class I A^{a}C^u) strains, which are concordant at the Class II loci, but discordant at both Class I loci. Furthermore, Class II^a *PVG.R23* rats share the Class I *A^u* allele with *BB-DP*, *LEW1.WR1* and *PVG.RT1^u* rats but do not develop diabetes in response to Poly IC. Thus, these studies do not show a requirement for a specific Class I^u allele in the diabetogenic response to Poly IC. Diabetes occurred with equal frequency in both males and females.

Lymphocytic insulitis and selective beta-cell destruction (Fig. 1) always accompanied diabetes. Diabetic rats had residual islet structures comprised only of non-beta endocrine cells (data not shown). The inflammatory cells infiltrating the islets of Langerhans were predominantly of the monocyte-macrophage lineage, with CD8$^+$ T cells making up the majority of insulitic T cells (Fig. 2). Thyroiditis occasionally accompanies spontaneous diabetes in the *BB* rat [17] but was not observed in any of the Poly IC-injected rats.

Poly IC treatment leads to the generation of diabetes-transferring spleen cells. Adoptive transfer studies were done to assess the role of T cells in Poly IC induced diabetes. Con A-activated spleen cells from spontaneously diabetic *BB* rats transfer diabetes into syngeneic as well as Class II-compatible recipients

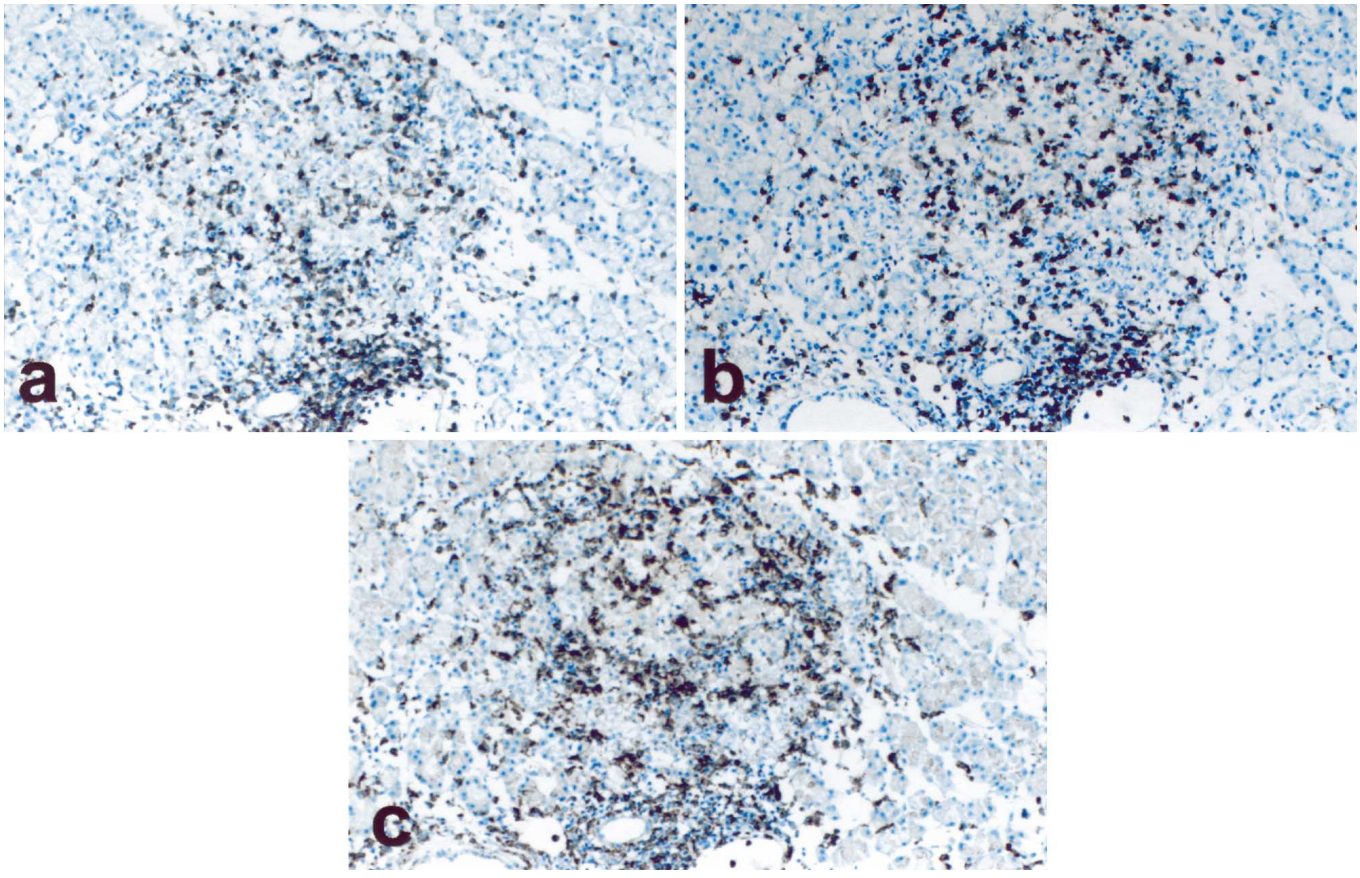

Fig. 2 A–C. Monocyte/macrophages and T cells infiltrate the islets of Langerhans in diabetes-susceptible Class II^u rats treated with Poly IC. Illustrated are immunoperoxidase-stained pancreatic sections of a *LEW1.WR1* rat treated for 9 days with Poly IC. Sections are stained with mABs directed against (**A**) OX-19 (pan T), (**B**) 341 (CD8 β-chain) and (**C**) ED-1 (monocytes, macrophages and dendritic cells). CD8$^+$ T cells make up the majority of islet-infiltrating lymphocytes

Table 2. Adoptive transfer of diabetes by Con A-activated spleen cells from Poly IC-treated rats

RT1	Donor	Recipient	Incidence of diabetes
u/u/a	*LEW1.WR1*	*LEW1.WR1*	17/17
u/u/u	*PVG.RT1*^{u}	*PVG.RT1*^{u}	7/7
u/a/a	*PVG.R23*	*PVG.R23*	0/5
c/c/c	*PVG.RT1*^{c}	*PVG.RT1*^{c}	0/10
u/u/u	*WF*	*DP/BB*	12/21
u/u/u	*WF*	*WF*	0/10
u/u/u	*WF*	*WAG/rnu*	4/6
u/u/u	*LOU*	*DP/BB*	0/5
u/u/u	*WAG*	*WAG/rnu*	4/6

After 2 weeks of Poly IC injections, spleen cells were Con A-activated in vitro and one spleen equivalent of activated cells was injected i.p. into 21 to 25-day-old syngeneic, *BB/Wor* diabetes-prone or *WAG/rnu* recipients. Blast transformation and cell viability (always > 90% by trypan blue) were not different between rat strains. Cell recipients were followed for 3 weeks for the development of glycosuria and hyperglycaemia (blood glucose $\geq$ 13.8 mmol/l)

[18]. Adoptive transfer of diabetes by Con A-activated spleen cells provides strong support for a T cell-mediated pathogenesis.

After 2 weeks of Poly IC or PBS injections, spleen cells were harvested and Con A activated in vitro. Poly IC-injected rats were not diabetic at the time of spleen harvesting. Activated cells were injected into naive syngeneic, *BB-DP/Wor*, or *WAG/rnu* recipients. The data in Table 2 demonstrate that Poly IC injections induce the generation of diabetes-transferring spleen cells in *Class II*^{u} LEW1.WR1, *PVG.RT1*^{u}, *WF* and *WAG* rats. Con A-activated spleen cells from Poly IC-injected *PVG.R23*, *PVG.RT1*^{c} and *LOU* rats did not transfer insulitis or diabetes. Finally, Con A-activated peripancreatic lymph node cells from Poly IC-treated *LEW1.WR1* rats transferred diabetes into 5 of 6 naive *LEW1.WR1* recipients (data not shown), also suggesting a role for T cells in disease transfer.

Surprisingly, Con A-activated *WF* spleen cells transferred insulitis and diabetes into *BB-DP* and *WAG/rnu* rats but not into *WF* rats. When pooled Con A-activated *WF* spleen cells were simultaneously transferred into both *BB-DP* and *WF* rats, only *BB-DP* recipients developed insulitis or diabetes. Thus, *WF* rats have diabetogenic T cells but those cells are ineffective in *WF* (but not *BB-DP* or *WAG/rnu*) hosts. *WF* rats do not, however, lack beta-cell target antigens because Con A-activated spleen cells

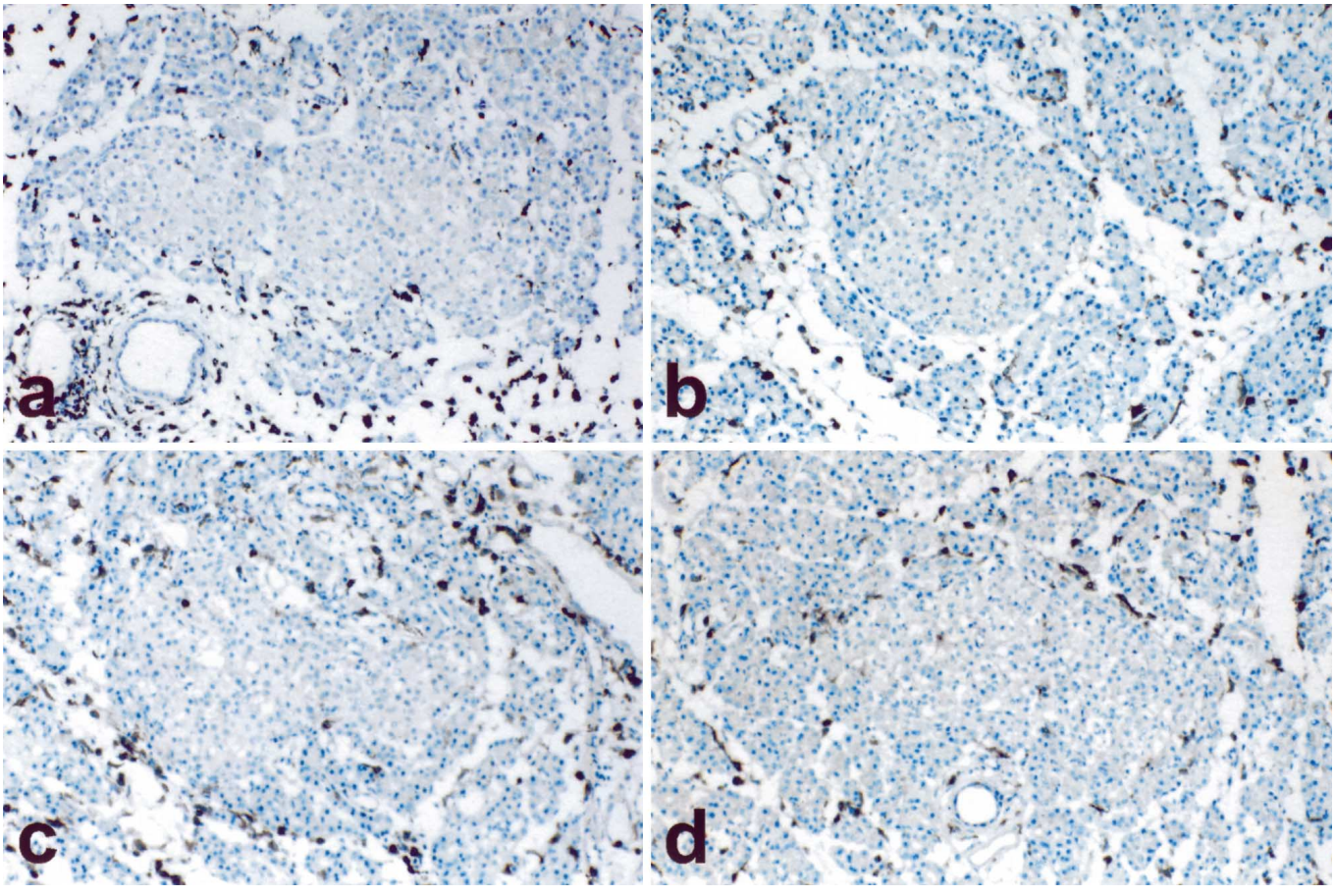

Fig. 3 A–C. Monocyte/macrophages and $CD8^+$ T cells do not infiltrate into the islets of Langerhans in diabetes-resistant rats treated with Poly IC. Illustrated are immunoperoxidase-stained pancreatic sections of Poly IC-treated rats. Sections are stained with mABs directed against CD8 (OX-8) (**B** and **C**) and ED-1 (**A** and **D**). Islets from PVG^c (**A** and **B**) and *WF* (**C** and **D**) rats are shown. Peri-insulitis but not intra-islet infiltrations are seen in diabetes-resistant strains. The *WF* rat was injected with Poly IC for 8 days and the PVG^c rat for 13 days

Table 3. The effect of 8 days of Poly IC injections on pancreatic morphology

Strain	Islet/ exocrine Class I	Endothelial VCAM-1	Endothelial ICAM-1	Infiltrating macs/ $CD8^+$T
LEW1.WR1	$3^+/3^+$	2^+	2^+	$3^+/2^+$
$PVG.RT1^u$	$3^+/3^+$	2^+	2^+	$3^+/2^+$
PVG.R23	$3^+/3^+$	1.5^+	2^+	$3^+/1^+$
$PVG.RT1^c$	$3^+/3^+$	1.5^+	2^+	$3^+/1^+$
WF	$3^+/3^+$	2^+	2^+	$3^+/2^+$
LOU	$3^+/3^+$	2^+	3^+	$3^+/3^+$

PBS-injected syngeneic rats served as base-line controls for immunostaining. Rats (n = 3–9) from each strain were analysed. Scoring was done on a scale of 0–3: 0 = constitutive levels (as seen in controls); 1+ = increased staining compared to controls; 2+ = moderately intense staining or cellular infiltration; 3+ = intense staining or extensive cellular infiltration. The mean value for each strain is given. The mononuclear cell infiltration scored in this table is restricted to the pancreatic interstitium

from diabetic *BB-DP* [19] or KRV-infected *BB-DR* [9] rats will transfer diabetes into *WF* recipients.

Poly IC injections up-regulate islet cell Class I and pancreatic endothelial ICAM-1 and VCAM-1 expression. To examine the proinflammatory changes which occur before the development of insulitis and diabetes, pancreatic tissues were removed after 5 and 8 days of injections. After 8 days of Poly IC, insulitis is occasionally present in diabetes-susceptible strains but is never present in diabetes-resistant strains. Immunoperoxidase stains were done on cryostat sections using monoclonal antibodies directed against rat Class I antigens, ICAM-1, VCAM-1, CD8, pan T cells and macrophage/dendritic cell lineages. Syngeneic PBS-injected rats served as baseline controls for immunostaining.

Poly IC injections given for 8 days induce islet and exocrine cell Class I hyperexpression (data not shown), up regulation of endothelial ICAM-1 and VCAM-1 and a concomitant T cell and macrophage infiltration of the pancreatic interstitium in all rat strains studied, including diabetes-resistant rats (Ta-

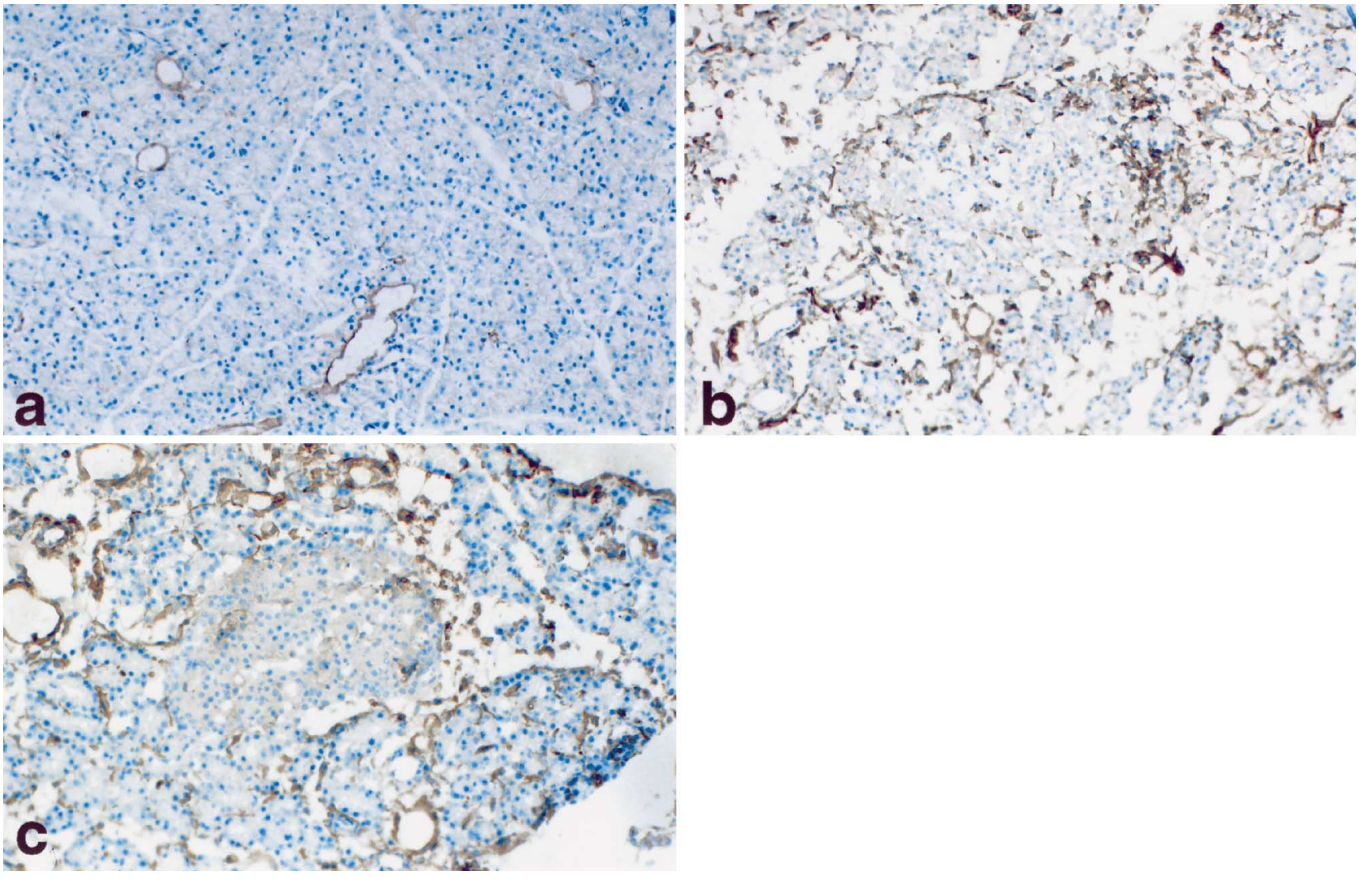

Fig. 4 A–C. Poly IC-treatment increases the numbers of ICAM-1$^+$ endothelial cells in both diabetes-susceptible and resistant rat strains. The intensity of ICAM-1 staining is also increased. Pictured are pancreatic sections stained by the immunoperoxidase method for ICAM-1 (CD54). **A** A control *LOU* rat injected with PBS for 8 days: endothelial cells exhibit constitutive levels of immunoreactive ICAM-1. **B** A diabetes-resistant *LOU* rat injected with Poly IC for 8 days. **C** A diabetes-susceptible *PVG^u* rat injected with Poly IC for 8 days. The induction of endothelial ICAM-1 is thus not sufficient for the development of Type I diabetes

ble 3, Fig. 2–5). As assessed by an examination of pancreatic histology after 5 and 8 days of Poly IC, the expression of islet cell Class I and endothelial VCAM-1 and the numbers of infiltrating macrophages increased progressively over time. Endothelial ICAM-1 was up regulated to the maximum after 5 days of Poly IC injections, with sustained high expression still seen after 8 days. The kinetics of Class I, ICAM-1 and VCAM-1 up regulation and macrophage infiltration were identical in all rat strains. The numbers of infiltrating T cells varied, however, between strains but did not correlate with susceptibility or resistance to disease induction. Finally, although the number of infiltrating CD8$^+$ T cells appeared to approach the number of OX-19$^+$ (pan) T cells (Fig. 2), we cannot rule out the presence of a minor population of infiltrating CD4$^+$ T cells. It is difficult to assess the numbers of CD4$^+$ T cells, because in the rat, macrophages and CD4$^+$ T cells share many of the same cell-surface markers, including CD4 [20].

Discussion

Our findings affirm that beta cell-autoreactive T cells and susceptibility to Type I diabetes are widely distributed in Class II^u haplotype rats. Autoimmunity is triggered in most Class II^u strains by injections of the IFN-α inducer Poly IC. Similarly, spontaneous diabetes in the rat has thus far been reported only in the Class II^u *BB-DP/Wor* [4–5] and *LETL* [6–7] strains. Susceptibility to diabetes in Class II^u, but not Class II^a or Class II^c rats, could be a consequence of the selective binding of the diabetogenic autoantigen to Class II^u molecules. Our studies thus support the peptide affinity model for the Class II genetic contribution to Type I diabetes susceptibility. The allelic polymorphisms of diabetes-associated Class II molecules dictate binding of specific beta cell peptides that result in T cell activation and beta-cell destruction [21].

Not all Class II^u haplotype rats developed insulitis and diabetes after Poly IC treatment, i. e. the *LOU*, *WAG* and *WF* rats. Thus, Class II binding of beta-cell peptide, islet cell Class I hyperexpression and increased leucocytic trafficking to the islets could be

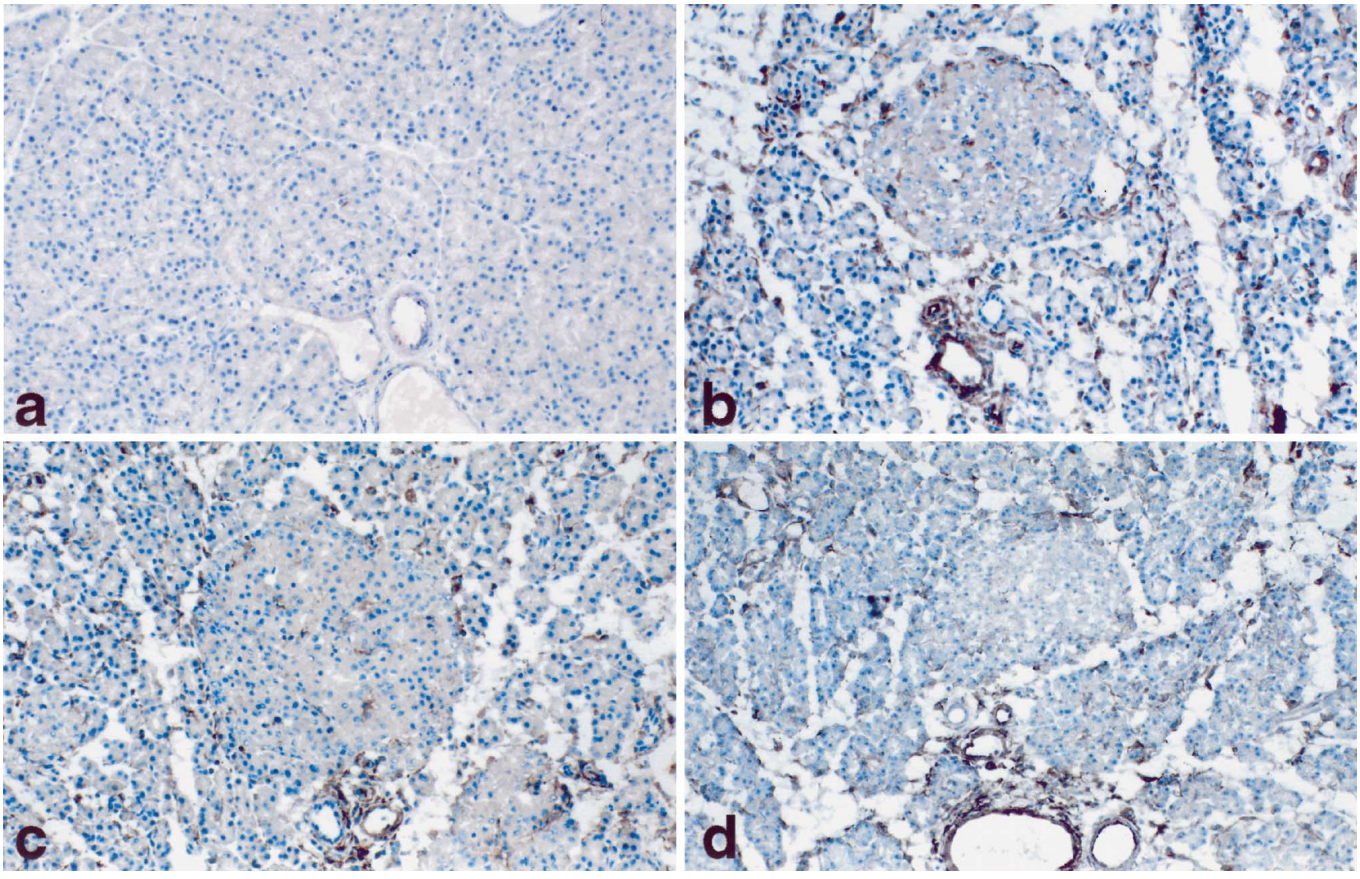

Fig. 5 A–D. Poly IC-treatment induces endothelial VCAM-1 in both diabetes-susceptible and resistant rat strains. Illustrated are pancreatic sections stained by the immunoperoxidase method for VCAM-1. **A** A control *PVG*^{u} rat injected with PBS for 8 days: endothelial VCAM-1 is not detected by immunostaining. **B** A diabetes-resistant *PVG*^{c} rat injected with Poly IC for 8 days. **C** A diabetes-resistant *PVG.R23* rat injected with Poly IC for 8 days. **D** A diabetes-susceptible *LEW1.WR1* rat treated with Poly IC for 8 days. The induction of endothelial VCAM-1 is not sufficient for the development of Type I diabetes

necessary, but are not sufficient, for the development of diabetes, even in the presence of susceptible Class II^{u} genes. The *LOU*, *WAG* and *WF* strains could possess a resistance allele at *Iddm 3* [22] or other diabetes-resistance genes. Given that most Class II^{u} haplotype strains are susceptible to experimentally induced diabetes and the incidence of spontaneous disease is very small, diabetes-resistance genes must be of paramount importance in preventing autoimmunity.

Poly IC injections also induced the generation of diabetes-transferring spleen cells in most Class II^{u} rats studied. This observation supports a pathogenic role for beta cell-autoreactive T cells in this model of autoimmune diabetes. The appearance of diabetes-transferring cells in *WF* and *WAG* rats was unexpected, given that they are largely resistant to the diabetogenic effects of Poly IC. Notably, T cells from *WF* rats can transfer diabetes into syngeneic and BB-DP rats after sensitisation by passaging through *BB-DP* recipients in vivo [23].

Poly IC treatment (and virus infections) could trigger autoimmunity by up regulating pancreatic endothelial adhesion receptors. Endothelial ICAM-1 mediates the firm adhesion of Mac-1^{+} and LFA-1^{+} leucocytes [24] and VCAM-1 mediates rolling and firm adhesion of $\alpha 4/\beta 1$-expressing (VLA-4^{+}) leucocytes (all leucocyte cell types except granulocytes) [24]. Indeed, up regulation of these molecules was accompanied by a large influx of macrophage/monocyte/dendritic cell lineages and T cells into the pancreatic interstitium. The increased leucocytic trafficking could result in enhanced presentation of beta cell antigen to autoreactive T cells in situ. Note that pancreatic tissue obtained from newly diagnosed human Type I diabetic patients also exhibited endothelial ICAM-1 up regulation [25–27]. Finally, the presence of a predominantly CD8^{+} T-cell infiltrate, coupled with islet cell Class I hyperexpression, suggests that cytotoxic T cells and Class I gene products are involved in the effector stages of beta-cell destruction.

Poly IC induces circulating IFN-α in *BB-DP/Wor* and *WF* rats [13], and this cytokine is believed to play a part in the pathogenesis of Type I diabetes. However, Poly IC increased serum IFN-α in *WF* rats to concentrations equal to or greater than those

found in *BB-DP* rats and induced diabetes in the *BB-DP*, but not the *WF* animals [13]. Interferon-α could be rate-limiting, however, if its cellular uptake or metabolism varies between strains. The role of IFN-α in other experimental models of Type I diabetes is contradictory. Transgenic beta-cell expression of IFN-α in mice can lead to insulitis and diabetes [28]. Islet expression of IFN-α precedes insulitis and diabetes in the *BB* rat [29]. Injections of recombinant IFN-α inhibit diabetes development, however, in the NOD mouse [30]. In the case of Poly IC, a cascade of cytokines, including IFN-α could be responsible for the induction of diabetes in susceptible strains of rat.

As reported, Poly IC injections do not induce T cell lymphopenia [16, 31]. In our studies, Poly IC-treated rats, whether diabetic or not, had normal numbers of total T cells and increased numbers of $CD8^+$ T cells in peripheral lymph nodes (data not shown). Thus, the diabetes-inducing effects of Poly IC cannot be ascribed to T-cell lymphopenia, a genetic susceptibility factor in the *BB-DP* [22] but not in the lymphopenic non-diabetic *BB-DR*/Edinburgh rat [32]. Of note is the recent report that *PVG.RT1^u* and *PVG.RT1^c* rats carrying the lymphopenia (*lyp*) gene do not develop diabetes ([33] and G. Butcher, personal communication). Clearly, lymphopenia and Class II^u alone are not permissive for the development of spontaneous disease, except in the case of the *BB-DP* rat.

We conclude that the requirements for autoimmune diabetes induction in the rat include at least three interacting elements: *RT1 Class II^u* genes, a T-cell repertoire containing beta cell-autoreactive T cells (as assessed by the ability to adoptively transfer diabetes into naive recipients) and a triggering event which breaks tolerance through the local up regulation of endothelial adhesion receptors, thus increasing mononuclear cell trafficking to the pancreas. Other genetic factors might also however, be necessary, because *WAG* and *WF* rats rarely develop diabetes in response to Poly IC, even though they possess Class II^u genes, beta-cell target molecules and diabetogenic T cells. Conversely, and perhaps more likely, resistance genes could be present in *WAG*, *WF* and *LOU* strains which prevent a diabetogenic response to Poly IC and other autoimmune stimuli. Finally, as double-stranded RNA is a potent inducer of diabetes in many rat strains, strong support is given to a role for Type I (α)-IFN-inducing viruses in the pathogenesis of Type I diabetes.

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