Class-II and IL2 receptor positive cells in the pancreas of NOD mice

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Summary. The aberrant expression of Class-II molecules on pancreatic B cells in Type 1 (insulin-dependent) diabetes is still a matter of debate. In order to verify if Class-II molecules are expressed on islet cells in the NOD mouse we have studied 21 female mice of different ages (5 to 22 weeks). Serial cryostat pancreas sections were stained with monoclonal rat antibodies against Class-II antigens (P7/7) and the IL2 receptor (AMT-13). Our results show no Class-II expression by endocrine cells at any age, whereas about 25–32% of mononuclear cells infiltrating the islets were Class-II posi-

tive, and only 6–9% were IL2 receptor positive. No staining, except of occasional tissue macrophages, was observed in the pancreas of BALB/c, CBA or B10.SCSN mice. Our data are in contrast with those recently published and therefore the reality of expression of Class-II molecules by islet cells of NOD mice should be viewed with caution.

Key words: Type 1 (insulin-dependent) diabetes, Class-II molecules, IL2 receptor, NOD mice, insulinitis, activated lymphocytes, lymphocyte subsets.

Type 1 (insulin-dependent) diabetes mellitus is an immune-mediated disease characterised both in humans and animal models by pancreatic lymphocytic infiltration [1–3]. In the latter the presence of islet cell antibodies has been reported together with other autoantibodies [4, 5]. Uncertainty persists concerning the extent and importance of aberrant expression of Class-II antigens on pancreatic B cells. This has been reported in newly diagnosed diabetic patients [6, 7] as well as in prediabetic BB/E rats [8]. A recent report [9] showed Class-II expression on B cells of diabetes prone NOD mice but also of non-diabetic B10.GD and BALB/c mice. Other reports suggest that Class-II antigens in mice are expressed only on monocytes, B lymphocytes and a few epithelial cells [10, 11] but not on islet cells. In humans these antigens are also expressed on activated T lymphocytes and are considered as a marker for T cell activation [12]. In rodents, however, their expression is much less frequent among lymphocytes [13].

We have investigated the expression of Class-II molecules on pancreatic islet cells in NOD and other non-diabetes prone strains of mice using the P7/7 MAb, while an anti-IL2 receptor (IL2R) MAb was used to detect activated T lymphocytes infiltrating the islets.

Materials and methods

Animals

Twenty-one female NOD mice (K^d, I-A^{NOD}, D^b [14]) were part of a colony kindly donated by E.H. Leiter (The Jackson Laboratory, Bar Harbor, Me, USA). They were killed at the age of 5, 9, 13, 17 and 22 weeks, the pancreata removed and immediately frozen in liquid nitrogen.

Five female BALB/c (H-2^d), two B10.SCSN (H-2^b) and two CBA (H-2^k) mice were used as control animals.

Fig. 1A–D. Immunoperoxidase staining of cryostat pancreas sections using P7/7 (A, B, C) and AMT-13 MAb (D). **A** An intact islet of a 5-week old NOD mouse. There are only a few tissue macrophages stained (arrows) (×225). **B** Infiltrated islet of a 22-week-old NOD mouse. Most of the mononuclear cells infiltrating the edge of the islet are Class-II positive but no staining is detectable on islet cells (×450). **C** An intact islet of a 15-week old BALB/c mouse. Staining is only detectable in a few tissue macrophages (arrows) (×225). **D** Infiltrated islet of a 22-week-old NOD mouse. Stained cells are activated lymphocytes expressing IL2 receptors (×360)

Table 1. Percentage of positive cells in the pancreas of NOD mice of different ages

Age (weeks) 5 ^a	9 ^b	13 ^c	17 ^b	22 ^c	
IL2R+ (AMT-13)	8.5±2.1	6.9±3.0	5.9±1.8	6.0± 2.2	7.9±4.6
Class-II+ (P7/7)	31.9±8.5	28.8±8.2	27.5±8.8	32.2±10.0	24.8±8.4

Data are mean ± SD. ^a n=3, ^b n=5, ^c n=4, ANOVA=NS

Immunohistochemistry

Serial cryostat pancreas sections (5 µm) were fixed in acetone for 30 min at room temperature and stained for 60 min with rat monoclonal antibodies P7/7 [10], culture supernatant at a 1:2 dilution in Tris Buffered Saline (TBS, pH 7.6), and AMT-13 (anti-IL2R, T. Diamanstein, Berlin, GDR), purified Ig from rat ascites at 5 µg/ml concentration. The P7/7 MAb recognises Class-II molecules of all tested haplotypes, including b, d and k [10]. Sections were washed three times in TBS prior to further incubation for 30 min with peroxidase conjugated rabbit anti-rat Ig (Dako P162) at a 1:50 dilution in TBS with 1:10 normal mouse serum. Positive cells were then revealed using diaminobenzidine and hydrogen peroxide [15] and counterstained with Mayer's haematoxylin.

Five different sections were examined per pancreas for a total of at least 10 different islets. In infiltrated islets at least 200 lymphocytes were counted per islet and positive lymphocytes were expressed as percentage of total lymphocytes per islet.

Results and discussion

The results of our study are summarised in Table 1. The percentage of Class-II positive infiltrating cells found in the pancreata of NOD mice ranged 25–32% between weeks 5 and 22 with no significant variations. In disagreement with recently published data by Hanafusa et al. [9] we have never observed Class-II expression on islet cells in NOD mice of any age (Figs. 1A, B). Two of the four mice of 22 weeks of age were newly diagnosed as diabetic and therefore, it is unlikely that these results can be explained by transient expression of Class-II on B cells present only at the time of onset of diabetes. Hanafusa et al. found Class-II positive islet cells (using mouse monoclonal anti-IA antibodies) in almost all pancreata from female mice and to a lesser extent in male NOD, in accordance with their incidence of diabetes. However, it is surprising that in most of these cases Class-II expression on islets preceded the lymphocytic infiltration, and also that BALB/c and B10.GD non-diabetes prone mice had I-A positive islet cells. The authors suggested three possible explanations for this phenomenon; but they did not consider the possibility of a cross-reaction of their monoclonal antibodies with other tissue antigens. Alternatively, the technical differences between our methods and those of Hanafusa et al. might account for the discordant results. While we used cryostat sections and rat MAbs these authors used Bouin's fixed and paraffin embedded material and mouse MAbs. We never observed Class-II expression on islet cells in

BALB/c (Fig. 1C), B10.SCSN and CBA mice, but tissue macrophages (which express I-A antigens) as well as some splenocytes (data not shown) were stained with P7/7.

In a previous report [10] it has been shown that P7/7 stains not only cells of haemopoietic origin but also a few epithelial cells. Furthermore, the administration of IFN-γ to B10 mice induced the expression of Class-II antigens in other epithelial cells but not in islet cells. Therefore it seems unlikely that B cells may express Class-II antigens which are not detected by our MAb.

Activated IL2R positive lymphocytes were constantly observed in all animals (Table 1 and Fig. 1D) but at any age their number was less than Class-II positive mononuclear cells.

Unlike human, mouse T lymphocytes do not express Class-II antigens following activation [11] with the exception, probably, of a small percentage of suppressor T cells [13, 16]. Therefore the I-A positive mononuclear cells infiltrating the islets in NOD mice cannot all be considered to be activated T lymphocytes, in contrast with what has been reported previously by Miyazaki et al. [2]. These cells mainly represent an heterogeneous population of monocytes and B lymphocytes as also suggested by Kanazawa et al. [5]. Furthermore, the presence of a large monocytic infiltration of the pancreas has also been reported in BB/W rats [17]. Since activated T lymphocytes and monocytes are distinct cell populations which play important roles in the pathogenesis of diabetes [17, 18] it is important that they are distinguished.

In conclusion, our data show that Class-II antigens in the pancreas of NOD mice are only expressed by infiltrating mononuclear cells which represent about 30% of total infiltrating cells. Activated IL2R positive T lymphocytes are less than 9% of infiltrating cells. Further investigations are required to clarify whether islet B cells can express Class-II antigens under any circumstances. At present our data suggest that they cannot; and in this respect the NOD mouse differs from the BB rat as an animal model for Type 1 diabetes.

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