Histol Histopathol (2002) 17: 393-401

**http://www.hh.um.es**

**Histology and Histopathology**

*Cellular and Molecular Biology*

# Abnormalities in dendritic cell and macrophage accumulation in the pancreas of nonobese diabetic (NOD) mice during the early neonatal period

**S. Charré[1], J.G.M. Rosmalen[2], C. Pelegri[3], V. Alves[1], P.J.M. Leenen[2], H.A. Drexhage[2] and F. Homo-Delarche[1]**

[1]CNRS UMR 8603, University Paris V, Necker Hospital, Paris, France, [2]Department of Immunology, Erasmus University, Rotterdam, The Netherlands and [3]Department of Physiology, Division IV, School of Pharmacy, University of Barcelona, Barcelona, Spain

**Summary.** Dendritic cell (DC), macrophage (Mø) and lymphocyte infiltrations have been observed in normal human perinatal pancreata, but have never been investigated so early in control mice. In type 1 diabetes-prone NOD mice, these cells are thought to infiltrate first the periphery of the islets of Langerhans around weaning before further islet infiltration and ß-cell destruction.

We quantified, during the first month of life, the numbers of DC (characterized by CD11c positivity and dendritic morphology), histiocyte-like Mø (characterized by ER-MP23 positivity) and Mø with scavenging potential (characterized by BM8 positivity) in C57BL/6, DBA/2 and BALB/c control, and NOD and lymphocyte-deficient NOD*scid* mouse pancreata.

First, $CD11c^+$ DC were present at low densities from birth onwards in control pancreata, while densities were higher in NOD and NOD*scid*. Second, high numbers of $BM8^+$ and ER-$MP23^+$ Mø were observed at birth in all strains investigated. After birth, particularly $BM8^+$ cells disappeared progressively in control strains, but not in NOD and NOD*scid*. Third, NOD mice also had more ER-$MP23^+$ Mø at birth compared to controls. Finally, DC and Mø localizations were similar in all strains, i.e., mostly as dispersed cells in perivascular, periductular, peri-islet areas and interlobular septa. The most remarkable finding was that particularly $BM8^+$ Mø, were seen at sites of islet neogenesis and predominantly at the duct-islet interface.

Our data showed that different types of APC were present in the pancreas during postnatal development in various control mouse strains and some differences were observed in NOD and NOD*scid* mice from birth onwards.

**Key words:** Macrophages, NOD, Neonatal pancreas development

*Offprint requests to:* Françoise Homo-Delarche, CNRS UMR 8603, Hôpital Necker, 161, rue de Sèvres, 75015 Paris, France. Fax: 33 1 44 49 06 76. e-mail: fhomodel@wanadoo.fr

## Introduction

Type 1 diabetes results from an autoimmune destruction of the ß cells in the pancreatic islets of Langerhans. In the spontaneous models of the disease, the NOD mouse and the Bio-Breeding (BB) rat, the accumulation of antigen-presenting cells (APC) around and in the islets of Langerhans is thought to be the earliest lesion, preceding progressive lymphocytic infiltration and ß-cell destruction (Voorbij et al., 1989; Bach, 1994; Jansen et al., 1994; Rossini et al., 1995). Dendritic cells (DC) are the APC par excellence and can be immunohistologically identified in the mouse by a dendritic morphology and CD11c positivity (Table 1). In NOD females, DC have been shown to accumulate around and within the islets of Langerhans from 3-5 weeks of age onwards (Rosmalen et al., 1997; Dalhen et al., 1998 ). During the same period, histiocyte-type Mø, which are immunohistochemically identifiable by their ER-MP23 positivity and normally present in pancreatic connective tissue, migrate to the periphery of the islets where some of them penetrate into the latter (Jansen et al., 1994). Such early accumulation of APC plays an important role in the development of insulitis, since prevention of a precocious influx of monocyte-derived cells stops the autoimmune process and protects against diabetes development (Hutchings et al., 1990). It is only after the early DC and ER-$MP23^+$ Mø accumulation, i.e., from 7-10 weeks of age onwards, that numerous T and B lymphocytes are recruited to the islets (Jansen et al., 1994). In NOD females, this is followed by an infiltration of $BM8^+$ Mø into the islets (Jansen et al., 1994). BM8 immunohistologically identifies histiocytes and Mø with a scavenging potential (Table 1). Similar sequences of infiltration events occur in the spontaneously developing autommune insulitis of the BB diabetes-prone rat (Voorbij et al., 1989).

Remarkably, DC, Mø and lymphocyte accumulation around or in the vicinity of islets is not solely a characteristic of autoimmune insulitis. Early studies noted a lymphocytic infiltration during normal human

fetal pancreas development concomitant with islet degeneration (Liu and Potter, 1962). More recent data confirmed the presence of DC, Mø and lymphocyte infiltrates in the exocrine pancreas and in the islet vicinity as a normal feature of fetal and neonatal human pancreata (Danilovs at al., 1982; Hofman et al., 1984; Motojima et al., 1989; Jansen et al., 1993).

In normal non-autoimmune mouse strain pancreata, lymphocytes are hardly present during the neonatal period, but the presence of DC and Mø has never been studied and quantified. We therefore analyzed from 1 day to 4 weeks of age, the number and localization of $CD11c^+$, ER-$MP23^+$ and $BM8^+$ cells in the pancreata of various control strains (C57BL/6, DBA/2 and BALB/c). We also approached the question of whether early postnatal DC and Mø infiltrates differ in mice with the NOD genetic background compared to control strains by evaluating their presence in NOD and NOD*scid* strains. The latter lacks functional lymphocytes and does not progress to lymphocytic insulitis and diabetes (Prochazka et al., 1992), but shares with the NOD mouse the early APC infiltration around and in the islets (Rosmalen et al., 2000b).

## Materials and methods

### *Animals*

NOD, NOD*scid*, C57BL/6, DBA/2 and BALB/c female mice were bred under specific pathogen-free conditions at the animal facilities of Hôpital Necker, Paris, France, as previously described (Amrani et al., 1998). The facilities and care followed the norms stipulated by the European Community. By 200 days of age, the incidence of diabetes in our NOD colony is 80% for females (Amrani et al., 1998). Mice of different strains were sacrificed on day 1, and at 1, 2 and 4 weeks of age for evaluation of DC and Mø infiltration.

### *Antibodies*

N418, a hamster anti-mouse CD11c antibody and ER-MP23, a rat anti-mouse Mø galactose-specific lectin (Table 1) were used as undiluted hybridoma culture supernatants (Jansen et al., 1993; Leenen et al., 1994, 1996). BM8, a rat anti-mouse antibody, that identifies a 125-kDa cell-surface protein (Table 1) was obtained from BMA Biomedicals AG, (Augst, Switzerland) and diluted 1/50 (Jansen et al., 1993; Leenen et al., 1994., 1996). BM8- and ER-MP23-binding was detected with horseradish peroxidase (HRP)-conjugated rabbit anti-rat immunoglobulins (Dako, Glostrup, Denmark) diluted 1/100. N418 was detected with HRP-conjugated goat anti-hamster immunoglobulins (Jackson Immuno-Research Laboratories, Inc., West Grove, PA, USA) diluted 1/200.

### *Immunohistochemistry*

Mice of different strains were killed by cervical dislocation after rapid retro-orbital bleeding. Their pancreata were removed, embedded in OCT (Tissue-Tek, Miles, Elkart, IN, USA) and frozen in n-hexane on dry ice-chilled alcohol. Tissues were stored at -80°C until immunohistochemistry was performed. Pancreas cryostat sections, 6 µm thick, were fixed for 10 min in acetone.

**Table 1.** Immunohistochemical staining characteristics of the N418, ER-MP23 and BM8 monoclonal antibodies.

| MONOCLONAL ANTIBODY | ANTIGEN | IMMUNOHISTOCHEMICAL STAINING |
|---|---|---|
| N418<br>hamster anti-mouse | $CD11^c$, an adhesion molecule belonging to the integrin family of adhesion molecules | Lymphoid tissue: Interdigitating dendritic cells in T-cell areas of primary and secondary lymphoid organs are strongly postive.<br>Peripheral dendritic cells (e.g. Langerhans cells) are not or barely positive. Activated Kuppfer cells are positive.<br>NOD Insulitis: Cells with a dendritic morphology, which infiltrate prior to the massive lymphocyte accumulation, are positive. Such cells also concentrate at the islet circumference. |
| ER-MP23<br>rat anti-mouse | A macrophage-specific 38-60 kDa lectin, binding sugar moities with a terminal galactose or N-acetyl galactosamine | Lymphoid tissue: Recent migrating macrophages into the secondary lymphoid tissues are positive, i.e., macrophages in sinuses and upper parts of the T-cell areas.<br>Peripheral tissue: Tissue-fixed macrophages are positive, particularly those located in the connective tissue (the so-called histiocytes).<br>NOD Insulitis: Macrophages concentrating at the islet circumference (prior to the massive lymphocytic accumulation) are positive. |
| BM-8<br>rat anti-mouse | A macrophage-specific 125 kDa surface protein with unknown function | Lymphoid tissue: Macrophages in the red pulp of the spleen are positive, as well as sinus macrophages in lymph nodes.<br>Peripheral tissue: Subset of histiocytes.<br>NOD Insulitis: Macrophages that infiltrate into the islets after the massive lymphocytic accumulation at the beginning of the islet destruction phase, are positive. |

References: Jansen et al. 1993; Leenen et al. 1994, 1996; Rosmalen et al. 2000 (for all three antibodies); Metlay et al. 1990 (for N418); Sato et al. 1998 (for ER-MP23) and Freudenberg et al. 1992 (for BM8).

After washing with phosphate-buffered saline containing 0.05% Tween-20 (Merck, Paris, France) (PBS/Tween), slides were incubated with primary monoclonal antibodies for 30 min at room temperature in a moist chamber. Subsequently, slides were washed twice with PBS/Tween and then incubated with secondary antibodies in the presence of 2% normal mouse serum for 30 min at room temperature. After washing with PBS/Tween, slides were incubated with 3-amino-9-ethylcarbazole (AEC, Sigma, Saint-Quentin-Fallavier, France), as the substrate, in 50 mM sodium acetate and 0.02 % $H_2O_2$, and washed in water after 3 min. Finally, slides were counterstained for 3 min in hematoxylin eosin (Merck), dehydrated in serially-graded ethanol baths, and mounted. For each series of pancreas sections, one slide was stained only with the second antibody as a control for endogenous peroxidase activity and non-specific antibody binding. A spleen or lymph node section was included as a positive control.

### *Immunohistochemical quantification*

The surface areas of the pancreata were assessed using a VIDAS-RT image analysis system (Kontron Elektronik GmbH/Carl Zeiss, Weesp, The Netherlands) and an objective magnification of x2. The surface areas of pancreatic sections were measured interactively and expressed in pixels. At this magnification, the size of one pixel is 0.00011955 $mm^2$. For all measurements, a total of 5 mice (one section per mouse) was analyzed per age-strain combination. The mean surface areas of the pancreas sections measured for the various strains were: 0.4-1.55 $mm^2$ at 1 day of age, 1.4-2.5 $mm^2$ at 1 week of age, 2.6-4.5 $mm^2$ at 2 weeks of age and 15-19 $mm^2$ at 4 weeks of age. The numbers of positive stained cells were counted separately by two different investigators and expressed per $mm^2$ of total pancreatic surface area of that particular section. The mean of these 2 measurements was taken and never exceeded 5%.

### *Statistical analyses*

Statistical significance for differences between cell numbers was determined by means of a two-way analysis of variance (ANOVA) followed by Newman-Keuls post-hoc analysis. The level of significance was set at $p<0.05$.

## Results

### *DC are present at low densities in control pancreata, as opposed to higher densities in NOD and NODscid mice from birth onwards*

During the postnatal period, $CD11c^+$ cells with a dendritic morphology were found in the pancreata of all strains investigated but at very low densities, i.e., 2-12 cells/$mm^2$ (Fig. 1). Although the numbers of $CD11c^+$ cells in a given strain remained relatively stable during the first 4 weeks of life, higher $CD11c^+$ cell densities were consistently observed from birth onwards in strains with the NOD genetic background ($p<10^{-6}$, as assessed by ANOVA). This effect was particularly evident, at 1 day of age, for NOD*scid* compared to C57BL/6 and DBA/2 mice (p=0.03 and 0.01, respectively, by post-hoc analyses) and tended towards significance for NOD versus DBA/2 and NOD*scid* versus BALB/c mice (p=0.06 and 0.08, respectively). At 4 weeks of age, $CD11c^+$ cell densities were significantly higher in NOD compared to C57BL/6 and DBA/2 mice (p=0.025 and 0.035, respectively). These latter differences correspond to the onset of classical insulitis in NOD mice (Jansen et al., 1994; Rosmalen et al., 1997; Dahlen et al., 1998).

Concerning their localization, the few $CD11c^+$ cells that were present were scattered throughout the wide connective tissue areas in the pancreata of all mouse strains at the youngest ages investigated (Fig. 2A-C). Thereafter, they were progressively found around vessels, ducts and islets, even in normal strains (Fig. 2 D-L). At 4 weeks of age, however, in NOD mice $CD11c^+$ cells could be seen inside the islets (Fig. 2), whereas they continued to surround the islets of control mice.

### *ER-MP23$^+$ histiocyte-type Mø are present at high densities in control neonatal pancreata, but at even higher numbers in NOD neonates*

Fig. 3 shows that, as compared to $CD11c^+$ cells, relatively high numbers of pancreatic ER-$MP23^+$ histiocyte-type Mø, i.e., 40-150 cells/$mm^2$ section, were detected in all strains during the first 3 weeks, but had declined sharply by 4 weeks of age. This age effect was highly significant ($p<10^{-6}$, ANOVA). Values for 4-week-old mice were significantly lower compared to those obtained at 1 day of age for C57BL/6, DBA/2, BALB/c

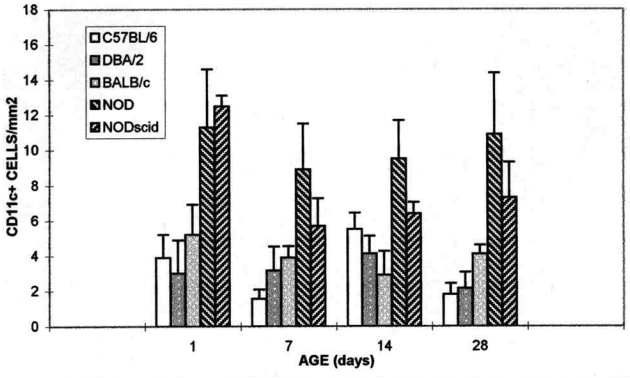


**Fig. 1.** Numbers of $CD11c^+$ DC in pancreata from various mouse strains as a function of age. Cells were counted and are expressed per $mm^2$ of total pancreatic surface area in each strain, as assessed by image analysis. A group of 5 mice (one section per mouse was analyzed per age-strain combination (mean values ± SEM)). More $CD11c^+$ DC are present in NOD and NOD*scid* mouse pancreata than in controls (strain effect: $p<10^{-6}$, as assessed by ANOVA).

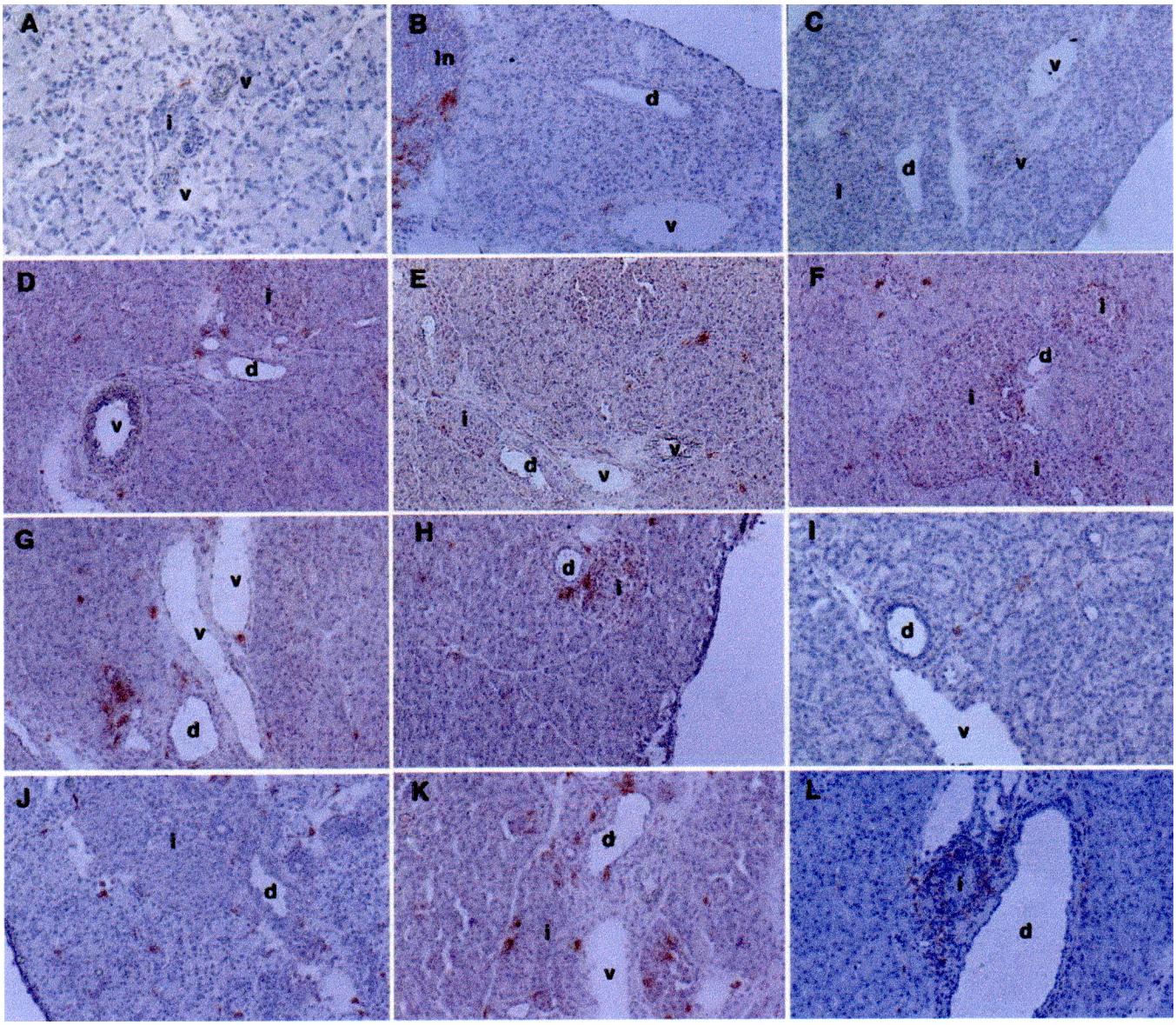


**Fig. 2.** Immunohistochemistry for CD11c$^+$ DC in control and NOD(*scid*) pancreata during the early postnatal period. **A-C.** 2-week-old control DBA/2 and (C) NOD*scid* pancreata. Note the CD11c$^+$ DC rarity except in peripancreatic lymph node (B). **D-F.** 4-week-old control DBA/2, BALB/c and C57BL/6 pancreata. Note the infiltration in perivascular, periductular and peri-islet areas. **G-I.** in 4-week-old NOD*scid* pancreata. Cells appear to be more abundant than in control pancreata but are similarly located. **J-L.** in 4-week-old NOD pancreata. DC are starting to invade the islet of Langerhans (L). d: duct; i: islet; ln: lymph node; v : vessel. A, x 440; B-L, x 280

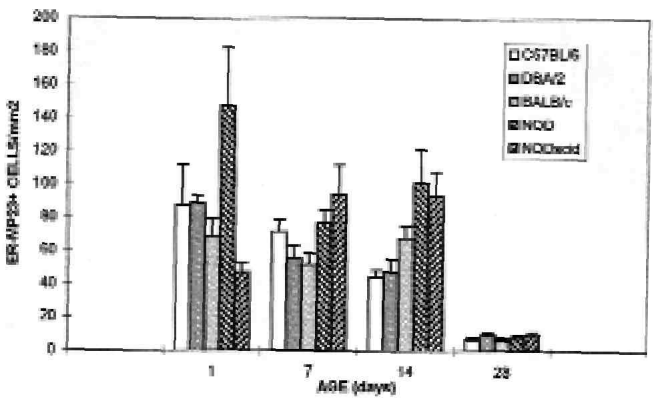


**Fig. 3.** Numbers of ER-MP23$^+$ Mø in pancreata from various mouse strains as a function of age. Results are expressed as in Figure 1. Although ER-MP23$^+$ Mø densities are high in control pancreata during the postnatal period, even more are present in 1-day-old NOD mice (p values from 0.02 to 0.0002 compared to all other strains, post-hoc analyses).

and NOD mice, at 1 week of age for C57BL/6, NOD and NOD*scid* mice, and at 2 weeks of age for NOD and NOD*scid* (p values from 0.05 to 0.0001, *post-hoc* analyses). Moreover, there were also differences between the strains (p=0.02, ANOVA): at 1 day of age, significantly more ER-MP23$^+$ cells were observed in NOD mice compared to all other strains, including NOD*scid* (p values from 0.02 to 0.0002, post-hoc analyses).

With regard to the localization, ER-MP23$^+$ histiocyte-type cells appeared to be rather dispersed within the pancreas at birth (Fig. 4A-C). Typically, these Mø were situated in the interlobular connective tissue separating the epithelial compartments, like CD11c$^+$ cells with dendritic morphology, but could also be found in peri-islet, periductular and perivascular areas (Fig. 4A,B). Beyond 1 week of age, ER-MP23$^+$ cells were also predominantly seen in perivascular, periductular and peri-islet areas (Fig. 4D,E,G,H,J,K), but also in interlobular areas (Fig. 4F,I,L) in all strains. As for

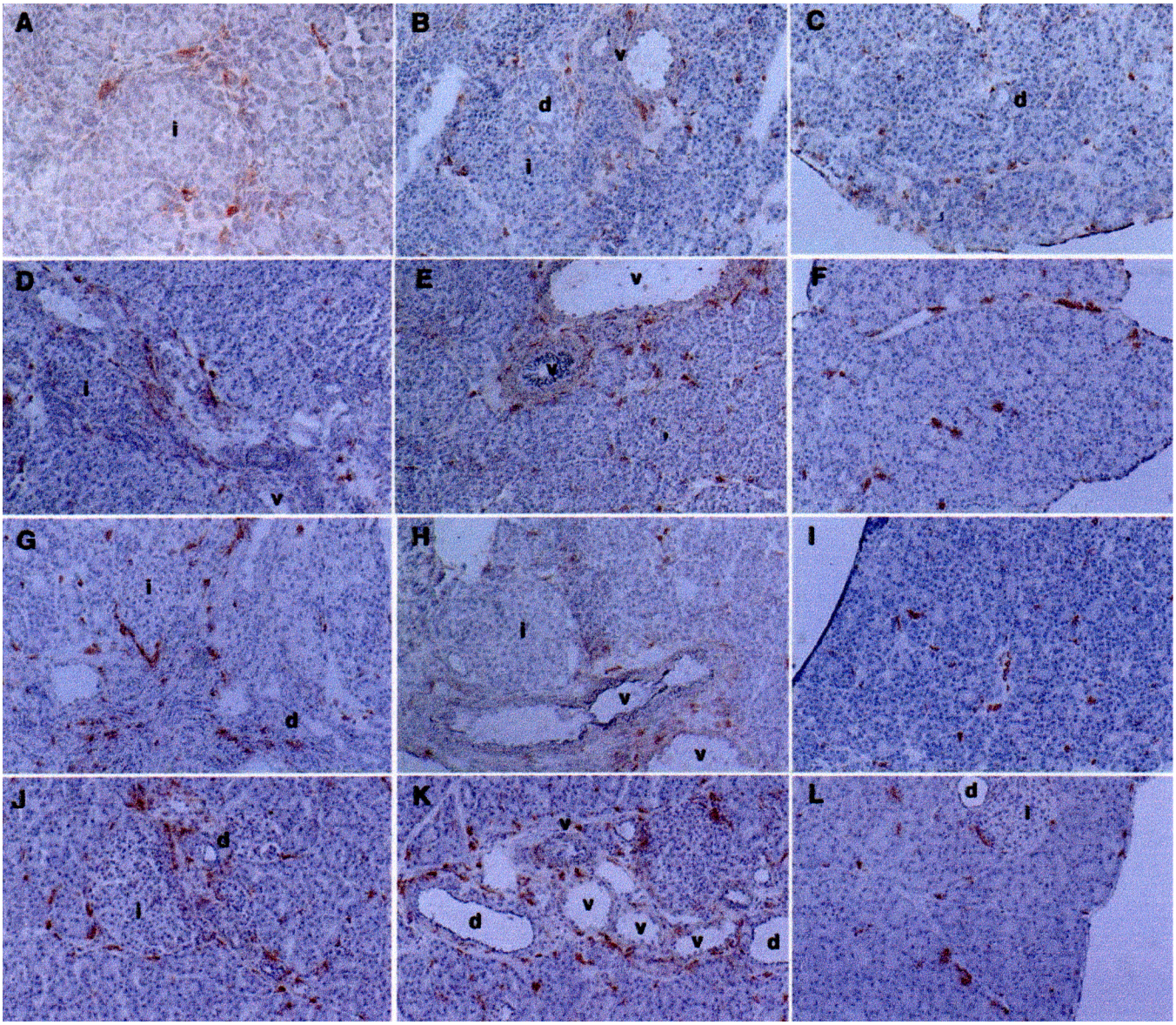


**Fig. 4.** Immunohistochemistry for ER-MP23$^+$ Mø in control and NOD(*scid*) pancreata during the early postnatal period. **A.** 1-day-old control C57BL/6 and **(B, C)** NOD pancreata. Note the presence in all strains of numerous cells in peri-islet **(A, B)**, peri-vascular and -ductular areas **(B)** or scattered **(C)**. **D-F.** 2-week-old control C57BL/6, BALB/c and DBA/2 pancreata, respectively. Abundant ER-MP23$^+$ Mø are present in localizations similar to those observed at birth. **G-I.** 2-week-old NOD*scid* **(G)** and NOD **(H, I)** pancreata. The cell localizations are the same as those observed in control mice, regardless of age. **J-L.** 4-week-old NOD **(J, K)** and NOD*scid* **(L)** pancreata. The cell infiltration reproduced events observed during normal early postnatal pancreas development. Abbreviations as in the legend to Fig 2. x 280

CD11c$^+$ DC, only in 4-week-old NOD mice, some ER-MP23$^+$ Mø could be found inside the islets (data not shown) (Jansen et al., 1994).

*The high densities of BM8$^+$ Mø in the neonatal period decrease in all strains after birth, but only slowly in NOD and NODscid mice*

BM8$^+$ Mø are also present in the pancreas during the early postnatal period. As for ER-MP23$^+$ Mø, relatively high densities of BM8$^+$ Mø were seen shortly after birth, i.e., 40-150 cells/mm$^2$ (Fig. 5). Their numbers declined significantly with increasing age, until they were hardly present ($p< 10^{-6}$, ANOVA). It should be noted that this decrease occurred earlier in control strains than in strains with the NOD genetic background, and was already significant between 1 day and 1 week of age for C57BL/6 and DBA/2 (p=0.01 and 0.0001, respectively, post-hoc analyses), and 1 and 2 weeks of age for BALB/c (p=0.01). In NOD and NOD*scid* mice, BM8$^+$ Mø densities also decreased significantly, but later, between 2 and 4 weeks of age (p=0.03 and 0.0001, respectively).

Moreover, at a given age, there were many significant differences between the various strains concerning BM8$^+$ Mø density. The most pertinent were the following: C57BL/6 pancreata contained the highest levels of BM8$^+$ Mø at 1 day and 1 week of age compared to all other strains (p values between 0.005 and 0.0001, *post-hoc* analyses), whereas 1-day-old NOD*scid* pancreata had the lowest values compared to all other strains, including the wildtype NOD (p values from 0.03 to 0.0001). Two-week-old NOD and NOD*scid* mice had significantly higher pancreatic BM8$^+$ Mø densities than control DBA/2 and BALB/c strains (p values between 0.04 and 0.001), but this effect was no longer significant at 4 weeks of age, when BM8$^+$ Mø numbers had decreased maximally in all strains.

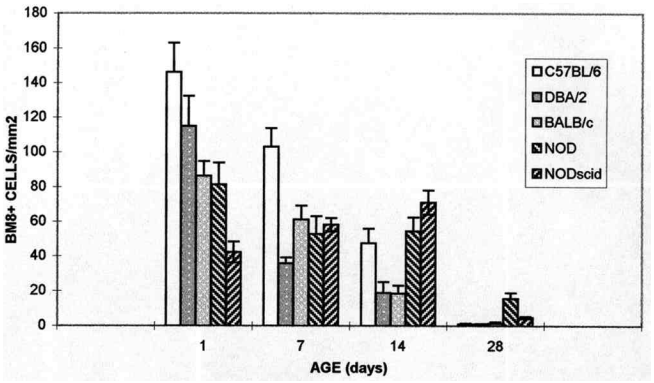


**Fig. 5.** Numbers of BM8$^+$ Mø in pancreata from various mouse strains as a function of age. Results are expressed as in Figure 1. Note that BM8$^+$ Mø numbers are highest at birth in the pancreata of all strains (age effect: $p<10^{-6}$, ANOVA), except NOD*scid* (p values from 0.03 to 0.0001 compared to other strains, post-hoc analyses).

Considering their localization, BM8$^+$ Mø were either scattered throughout the pancreas or already grouped together in peri-islet or peri-acinar areas at birth (Fig. 6 A-C). An overlap between the BM8$^+$ and ER-MP23$^+$ Mø populations may exist, yet our data on serial sections indicate that such overlap is very limited at birth (data not shown). Thereafter, they were commonly observed in perivascular, periductular and peri-islet areas and septa in all strains (Fig. 6D-L). Remarkably, BM8$^+$ Mø were sometimes seen at the sites of islet neogenesis, i.e., the differentiation of islets from ducts (Fig. 6G,H). BM8$^+$ Mø surrounded the ducts and the islets in an almost continuous line and they appeared to accumulate at the interface between the two structures. Such images were regularly seen in all strains until 4 weeks of age.

## Discussion

The results of this study indicate that relatively high numbers of Mø and relatively low numbers of DC are present in the connective tissue of normal newborn non-autoimmune mouse pancreata. Mø densities decreased sharply after weaning. The presence and localization of scavenger-type Mø (BM8$^+$) were the most remarkable. High densities of these cells were present at birth and then progressively declined during the first month of life. At 4 weeks of age, i.e. after weaning, BM8$^+$ Mø were hardly detectable anymore, confirming previously reported findings (Jansen et al., 1994). Histiocyte-type Mø (ER-MP23$^+$) were also present at relatively high levels in normal strains from birth onwards, but their numbers were quite similar for all these strains and remained stable during the first 2 weeks of life. A decline was observed at 4 weeks of age, but low numbers of ER-MP23$^+$ Mø persisted, a finding that is consistent with their presence in the normal adult mouse pancreas (Jansen et al., 1994).

What could be the reason for this early relatively high presence of Mø in neonatal (1 day) pancreata of normal mice? It is worth noting that the decline in the numbers of these cells in the following 4 weeks coincides with the completion of postnatal pancreas development (Bouwens et al., 1994; Bouwens and Kloppel, 1996; Scaglia et al., 1997). Tissue remodeling phenomena (islet neogenesis, ß-cell replication and apoptosis of various cell types) are at work in the pancreata of normal rodents, and particularly in mice until weaning (Bouwens et al., 1994; Scaglia et al., 1997). At present, little is known about the mechanisms involved in such processes of islet neogenesis and those which lead to the detachment of the islet from the duct and the rupture of the basement membrane surrounding both structures (Pictet and Ruter, 1972). The processes through which pancreatic interlobular septa develop are also unknown. Macrophages are likely involved in these developmental processes, since they have a well-recognized role in tissue remodeling (synthesis and degradation of extracellular matrix) and apoptosis induction (Nathan, 1987; Ibrahim et al., 1995; Aliprantis

et al., 1996). Moreover, Mø are able to produce many of the cytokines and growth factors known to be implicated in normal pancreas development (Bouwens and Kloppel, 1996; Sarvetnick, 1997). Also Mø play a major role in the processes of pancreatic degeneration and regeneration which take place after duct ligation-induced pancreatitis (Walker et al., 1992; Yamaguchi et al., 1993). It is furthermore worth noting that macrophages in association with eosinophils have recently been shown to play a key role during normal postnatal mouse mammary gland development, regulating branching morphogenesis (Gouon-Evans et al., 2000). We therefore consider our observation in this study that Mø were particularly present at tissue-remodeling locations during the postnatal period, both in control mice as well as in mice with the NOD genetic background, as a strong indication that these cells are at least partially involved in islet development from ducts.

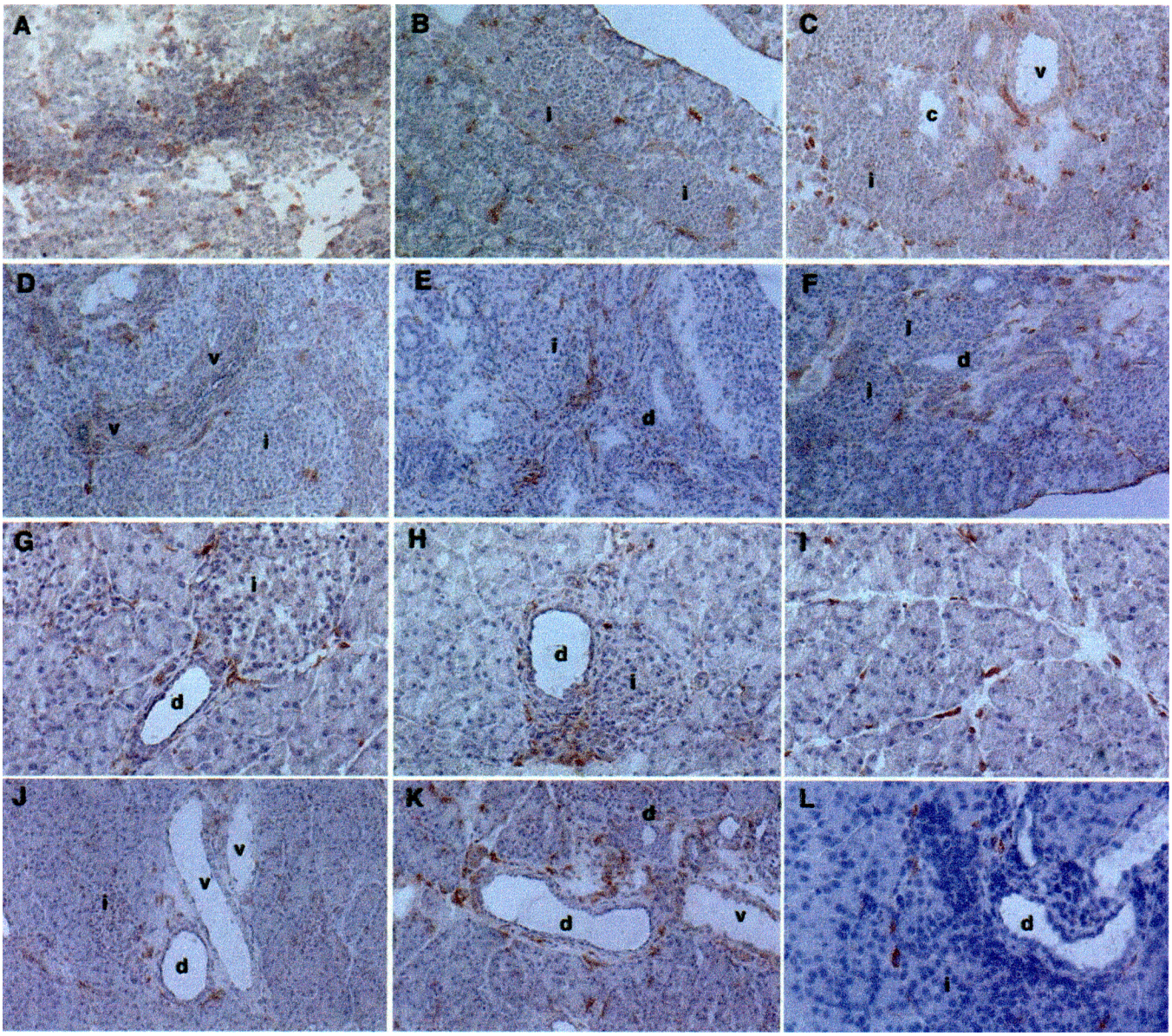


**Fig. 6.** Immunohistochemistry for BM8$^+$ Mø in control and NOD pancreata during early postnatal pancreas development **(A-C)** in 1-day-old control C57BL/6 and NOD **(B, C)** pancreata. Cells are abundant and either scattered **(A)** or in peri-islet, peri-ductular and -vascular areas (**B, C**). **D-F.** 2-week-old control C57BL/6 **(D)**, NOD*scid* **(E)** and NOD **(F)** pancreata. Cell localizations are similar in all strains. **G-I.** 4-week-old control C57BL/6 pancreata; BM8$^+$ Mø appear to accumulate at duct-islet interfaces and are present in septa. **J-L.** 4-week-old NOD*scid* **(J)** and NOD **(K, L)** pancreata. Cell localizatons are similar in control and NOD strains during the entire early postnatal period. Abbreviations as in the legend to Fig 2. A-F, J, K, x 280; G-I, L, x 440

A second intriguing finding of this study is that NOD and NOD*scid* mice already had minor quantitative abnormalities in pancreatic Mø and DC infiltration from birth onwards, i.e., long before lymphocytic peri-insulitis becomes evident in NOD mice by light microscopy (from 5-7 weeks of age onwards). Indeed, DC, characterized by their dendritic morphology and CD11c positivity, were detected in control strains at low and stable numbers during the first month of life, but were consistently more abundant in NOD and NOD*scid* pancreata. In this regard, it should be noted that an active autoimmune reaction has already been postulated in the NOD fetus based on an *in utero* T-cell activation (Wilson and de Luca, 1997). Such an early autoimmune reaction might perhaps be responsible for the high local APC densities in the neonatal NOD mouse pancreas. However, NOD*scid* mice do not possess functional lymphocytes and the higher number of DC seen in their pancreata can not be due to lymphocytic autoreactivty. Several genetically-determined defects in APC diffrentiation/function have been described in NOD mice (Lety et al., 1992; Serreze et al., 1993; Luan et al., 1996; Piganelli et al., 1998; Radosevic et al., 1999) and they might influence the migratory kinetics of NOD strain DC, thus explaining their higher accumulation in the neonatal pancreas.

Another strain difference in terms of pancreatic cell densities at birth was seen between NOD and NOD*scid* mice: the latter had significantly fewer pancreatic ER-MP23$^+$ and BM8$^+$ Mø compared to NOD mice at 1 day of age. The reason for this lower number of Mø is unclear. Perhaps, Mø number and/or function could be decreased in NOD*scid* mice due to a state of relative inactivation of the Mø system resulting from the absence of functional lymphocytes. This might also be indicated by the reported finding that Mø isolated from NOD*scid* islets are much less effective in stimulating diabetogenic T-cell clones than those isolated from NOD islets (Shimizu et al., 1995). In addition, *in vivo* transfer of T-cell clones improves the antigen-presenting function of NOD*scid* Mø (Shimizu et al., 1995).

Finally, another significant strain difference between control mice and mice with the NOD genetic background concerns the kinetics of cell disappearance. In particular, NOD and NOD*scid* mice maintained stable numbers of BM8$^+$ Mø during the first 2 weeks of life, while the later decline was not as progressive as that observed in control strains. The reason for the abnormal BM8$^+$ Mø kinetics remains unknown. First, it may be -as the higher presence of DC- a sign of enhanced autoimmune reactivity in the neonatal NOD mouse as mentioned above. Second, if BM8$^+$ Mø are involved in islet neogenesis and tissue remodeling (vide supra), then the abnormal kinetics may indicate an abnormal islet development in strains with the NOD background. Indeed, we have previously reported on various growth and function abnormalities of NOD islets prior to peri-insulitis development (Homo-Delarche, 1997; Amrani et al., 1998; Pelegri et al., 2001). Third, intrinsic DC and Mø abnormalities exist in NOD strains, such as a defective FcγRII gene expression, a deficient differentiation from bone-marrow precursors, a decreased cytokine secretion and an enhanced arachidonic acid metabolism (Lety et al., 1992; Serreze et al., 1993; Luan et al., 1996; Piganelli et al., 1998; Radosevic et al., 1999). These abnormalities may also influence the migration kinetics of BM8$^+$ cells.

In conclusion, this study shows various abnormalities in APC accumulation in the postnatal developing pancreas of mouse strains with the NOD genetic background, far before the start of the classical lymphocytic peri-insulitis, which usually starts after 5-7 weeks of age in NOD mice. The functional significance of these cells remains to be elucidated, but in light of their localization in all strains, it could be suggested that they participate to pre- and postnatal pancreas morphogenesis and, particularly, islet neogenesis from duct. Moreover, it is not excluded that, at birth, the differences between NOD and control strains are linked to disturbances of glucose homeostasis in NOD mothers and those observed later to a different islet behavior to lactation and/or weaning.

*Acknowledgements.* The authors wish to thank I. Cissé and J. Coulaud for technical assistance and Ms. C. Slama for excellent secretarial work. This research was supported by grants from CNRS, Université Paris V, Fondation de France, Alfediam (associated with Lilly Laboratories), NWO-INSERM, BIOMED ("Betimmune"), 5th PCRD (Monodiab) and the Diabetes Fonds Nederland.

## References

Aliprantis A.O., Diez-Roux G., Mulder L.C.F., Zychlinsky A. and Lang R.A. (1996). Do macrophages kill through apoptosis? Immunol. Today 17, 573-576.

Amrani A., Durant S., Throsby M., Coulaud J., Dardenne M. and Homo-Delarche F. (1998). Glucose homeostasis in the nonobese diabetic mouse at the prediabetic stage. Endocrinology 139, 1115-1124.

Bach J.F. (1994). Insulin-dependent diabetes mellitus as an autoimmune disease. Endocr. Rev. 15, 516-542.

Bouwens L. and Kloppel G. (1996). Islet cell neogenesis in the pancreas. Virchows Arch. 427, 553-560.

Bouwens L., Wang R.N., de Blay E., Pipeleers D.G. and Kloppel G. (1994). Cytokeratins as markers of ductal cell differentiation and islet neogenesis in the neonatal rat pancreas. Diabetes 43, 1279-1283.

Dahlen E., Dawe K., Ohlsson L. and Hedlund G. (1998). Dendritic cells and macrophages are the first and major producers of TNF-alpha in pancreatic islets in the nonobese diabetic mouse. J. Immunol. 160, 3585-3593.

Danilovs J.A., Hofman F.M., Taylor C.R. and Brown J. (1982). Expression of HLA-DR antigens in human fetal pancreas tissue. Diabetes 31 (suppl. 4), 23-29.

Gouon-Evans V., Rothenberg M.E. and Pollard J.W. (2000). Postnatal mammary gland development requires macrophages and eosinophils. Development 127, 2269-2282.

Hofman F.M., Danilovs J.A. and Taylor C.R. (1984). HLA-DR (Ia)-positive dendritic-like cells in human fetal nonlymphoid tissues.

Transplantation 37, 590-594.

Homo-Delarche F. (1997). Beta-cell behaviour during the prediabetic stage. Part II. Non-insulin- dependent and insulin-dependent diabetes mellitus. Diabetes Metab. 23, 473-505.

Hutchings P., Rosen H., O'Reilly L., Simpson E., Gordon S. and Cooke A. (1990). Transfer of diabetes in mice prevented by blockade of adhesion-promoting receptor on macrophages. Nature 348, 639-642.

Ibrahim M.A., Chain B.M. and Katz D.R. (1995). The injured cell: the role of the dendritic cell system as a sentinel receptor pathway. Immunol. Today 16, 181-186.

Jansen A., Voorbij H.A., Jeucken P.H.M., Bruining G.J., Hooijkaas H. and Drexhage H.A. (1993). An immunohistochemical study on organized lymphoid cell infiltrates in fetal and neonatal pancreases. A comparison with similar infiltrates found in the pancreas of a diabetic infant. Autoimmunity 15, 31-38.

Jansen A., Homo-Delarche F., Hooijkaas H., Leenen P.J., Dardenne M. and Drexhage H.A. (1994). Immunohistochemical characterization of monocytes-macrophages and dendritic cells involved in the initiation of the insulitis and beta-cell destruction in NOD mice. Diabetes 43, 667-675.

Freudenberg N., Piotraschke J., Galanos C., Sorg C., Askaryar F.A., Klosa B., Usener H.U., and Freudenberg M.A. (1992). The role of macrophages in the uptake of endotoxin by the mouse liver. Virchows Arch. (B) 61, 343-349.

Leenen P.J.M., de Bruijn M.F.T.R., Voerman J.S.A., Campbell P.A. and Van Ewijk W. (1994). Markers of mouse macrophage development detected by monoclonal antibodies. J. Immunol. Methods 174, 5-19.

Leenen P.J.M., Kraal G. and Dijkstra C.D. (1996). Markers of rodent myeloid cells. In: Immunology methods manual. The comprehensive sourcebook of techniques. Lefkovits I. (ed). Academic Press. San Diego. pp 2467-2495.

Lety M.A., Coulaud J., Bens M., Dardenne M. and Homo-Delarche F. (1992). Enhanced metabolism of arachidonic acid by macrophages from nonobese diabetic (NOD) mice. Clin. Immunol. Immunopathol. 64, 188-196.

Liu H.M. and Potter E.L. (1962). Development of the human pancreas. Arch. Pathol. 74, 53-66.

Luan J.J., Monteiro R.C., Sautes C., Fluteau G., Eloy L., Fridman W.H., Bach J.F. and Garchon H.J. (1996). Defective Fc gamma RII gene expression in macrophages of NOD mice: genetic linkage with up-regulation of IgG1 and IgG2b in serum. J. Immunol. 157, 4707-4716.

Metlay J.P., Witmer-Pack M.D., Agger R., Crowley M.T., Lawless D. and Steinman R.M. (1990). The distinct leukocyte integrins of mouse spleen dendritic cells as identified with new hamster monoclonal antibodies. J. Exp. Med. 171, 1753-1771.

Motojima K., Matsuo S. and Mullen Y. (1989). DR antigen expression on vascular endothelium and duct epithelium in fresh or cultured human fetal pancreata in the presence of gamma-interferon. Transplantation 48, 1022-1026.

Nathan C.F. (1987). Secretory products of macrophages. J. Clin. Invest. 79, 319-326.

Pelegri C., Rosmalen J.G.M., Durant S., Throsby M., Alves V., Coulaud A., Esling A., Pleau J.M., Drexhage H.A. and Homo-Delarche F. (2001). Islet endocrine cell behavior from birth onwards in mice with the nonobese diabetic genetic background. Mol. Med. 7, 311-319.

Piganelli J.D., Martin T. and Haskins K. (1998). Splenic macrophages from the NOD mouse are defective in the ability to present antigen. Diabetes 47, 1212-1218.

Pictet R. and Rutter W.J. (1972). Development of the embryonic endocrine pancreas. In: Handbook of physiology, Section 7: Endocrinology, Vol. 1. Geiger S.R (ed). Waverley Press. pp 25-66.

Prochazka M., Gaskins H.R., Schulz L.D. and Leiter E.H. (1992). The nonobese diabetic *scid* mouse: model for spontaneous thymomagenesis associated with immunodeficiency. Proc. Natl. Acad. Sci. USA 89, 3290-3294.

Radosevic K., Casteels K.M., Mathieu C., Van Ewijk W., Drexhage H.A. and Leenen P.J. (1999). Splenic dendritic cells from the nonobese diabetic mouse induse a prolonged proliferation of syngeneic T cells. A role for an impaired apoptosis of NOD T cells? J. Autoimmunity 13, 373-382.

Rosmalen J.G., Leenen P.J., Katz J.D., Voerman J.S. and Drexhage H.A. (1997). Dendritic cells in the autoimmune insulitis in NOD mouse models of diabetes. Adv. Exp. Med. Biol. 417, 291-297.

Rosmalen J.G., Martin T., Dobbs C., Voerman J.S., Drexhage H.A., Haskins K. and Leenen P.J. (2000a). Subsets of macrophages and dendritic cells in nonobese diabetic mouse pancreatic inflammatory infiltrates: correlation with the development of diabetes. Lab. Invest 80, 23-30.

Rosmalen J.G.M., Homo-Delarche F., Durant S., Kap M., Leenen P.J.M. and Drexhage H.A. (2000b). Islet abnormalities associated with an early influx of dendritic cells and macrophages in NOD and NOD*scid* mice. Lab. Invest. 80, 769-777.

Rossini A.A., Handler E.S., Mordes J.P. and Greiner D.L. (1995). Animal models of human disease. Human autoimmune diabetes mellitus: lessons from BB rats and NOD mice-Caveat Emptor. Clin. Immunol. Immunopathol. 74, 2-9.

Sarvetnik N. (1997) Pancreatic growth and regeneration. Karger Landes Systems. Basel.

Sato K., Imai Y., and Irimura T. (1998). Contribution of dermal macrophage trafficking in the sensitization phase of contact hypersensitivity. J. Immunol. 161, 6835-6844.

Scaglia L., Cahill C.J., Finegood.D.T. and Bonner-Weir S. (1997). Apoptosis participates in the remodeling of the endocrine pancreas in the neonatal rat. Endocrinology 138, 1736-1741.

Serreze D.V., Gaskins H.R. and Leiter E.H. (1993). Defects in the differentiation and function of antigen-presenting cells in NOD/Lt mice. J. Immunol. 150, 2534-2543.

Shimizu J., Carrasco-Marin E., Kanagawa O. and Unanue E.R. (1995). Relationship between ß cell injury and antigen presentation in NOD mice. J. Immunol. 155, 4095-4099.

Voorbij H.A., Jeucken P.H., Kabel P.J., de Haan M. and Drexhage H.A. (1989). Dendritic cells and scavenger macrophages in pancreatic islets of prediabetic BB rats. Diabetes 38, 1623-1629.

Walker N.I., Winterford C.M. and Kerr J.F. (1992). Ultrastructure of the rat pancreas after experimental duct ligation. II. Duct and stromal cell proliferation, differentiation and deletion. Pancreas 7, 420-434.

Wilson S.S. and de Luca D. (1997). NOD fetal thymus organ culture: an in vitro model for the development of T cells involved in IDDM. J. Autoimmunity 10, 461-472.

Yamaguchi Y., Matsuno K., Goto M. and Ogawa M. (1993). In situ kinetics of acinar, duct, and inflammatory cells induct ligation-induced pancreatitis in rats. Gastroenterology 104, 1498-1506.

Accepted December 10, 2001