Novartis Foundation Symposium 292

DEFINING OPTIMAL IMMUNOTHERAPIES FOR TYPE 1 DIABETES

WILEY-BLACKWELL
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Contents

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Chair's introduction

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Diabetes is a disease where we still have many gaps in our knowledge. It is a special disease because we can't access the organ very well, especially during the prediabetic phase in humans. Perhaps by linking animal studies with *in vitro* studies of human cells and then actual human studies we can close some of these gaps during this meeting. This pertains to both the basic pathogenesis of the disease as well as clinical translations.

There are many areas that are important to me in this field. I want to learn more about how the human disease actually comes together. I want to understand the kinetics. There are certain things that continue to puzzle me: I don't understand how an immune-mediated disease is sustained for such a long time (in some cases the prediabetic phase can last more than seven years). How can it be that cells are continuously regenerated to attack islets in this chronic fashion? That a comparatively low-grade inflammatory immunological process can continue like this for several years puzzles me.

Understanding these types of kinetics will not only translate into understanding the pathogenesis, but also devising an optimal therapy: for example, we do not know for how long we would have to stop aggressive cells for in order to circumvent recurrence of disease. Does immunosuppressive or immune modulatory therapy have to be administered continuously, even if bystander regulation and other immunological control mechanisms that can be self-sustained by autoantigens are being invoked? Here we should discuss these issues, and others, for example with the question of the number of important autoantigens in type 1 diabetes: is there just one antigenic 'driver'pathway? I would also like to see parallels made with other diseases, where applicable, and we have therefore invited speakers whose main expertise is in multiple scerosis and other autoimmune disorders.

Retrospectively, this conference turned out to be a treat in many respects even for those who would consider themselves to be seasoned investigators in the pathogenesis of type 1 diabetes. We uncovered crucial 'forgotten' human data sets that should be revisited and expanded, we learned much more about the human aspects of type 1 diabetes pathogenesis which will be important to properly adjust current animal models, and we better comprehended crucial therapeutic and kinetic issues of the disease.

Pancreatic pathology in type 1 diabetes in human

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Abstract. In type 1 autoimmune diabetes there is a selective destruction of insulin-secreting β cells. Around the time of clinical presentation, insulitis, a chronic inflammatory infiltrate of the islets affecting primarily insulin containing islets, is present in the majority of cases. The inflammatory infiltrate consists primarily of T lymphocytes; CD8 cells outnumber CD4 cells, there are fewer B lymphocytes and macrophages are relatively scarce. β cell death may involve the Fas apoptotic pathway since they have been shown to express Fas, infiltrating T lymphocytes express Fas-L and apoptotic β cells have been described. Hyperexpression of class I MHC by all the endocrine cells in many insulin-containing islets is a well recognized phenomenon, characteristic of the disease. It has been argued that this is an earlier event than insulitis within a given islet and appears to be due to secretion of interferon α by β cells within that islet. A recent study has found evidence of Coxsackie virus infection in β cells in three out of six pancreases of patients with recent-onset type 1 diabetes. Coxsackie viruses are known to induce interferon α secretion by β cells and this could initiate the sequence of events that culminates in their autoimmune destruction.

2008 Defining optimal immunotherapies for type 1 diabetes. Wiley, Chichester (Novartis Foundation Symposium 292) p 2–18

There are a number of different ways of obtaining pancreas specimens from patients with type 1 diabetes. Historically, the most common source was retrospective collections of autopsy pancreases from children who had died around the time of clinical diagnosis (Foulis et al 1986, Gepts 1965). The disadvantage of this approach was that there was usually a degree of autolysis in the tissues and the pancreas would almost certainly have been fixed in formalin and paraffin embedded. These factors and the lack of access to peripheral blood of the patient limited the range of possible studies on these pancreases.

A radical departure from this historical practice has been the approach of the group from Osaka. They performed laparoscopic pancreatic biopsies on patients who had been diagnosed with type 1 diabetes in the previous three months. A great range of tests has been done on this tissue and the results have been correlated with clinical findings. The disadvantage is that the biopsies were small

(20–30 mg) leading to a possible sampling problem. Thus the biopsies of three out of the first seven patients had no insulin-containing islets (Hanafusa et al 1990). While pancreatic biopsy has proven to be safe, no other research group has adopted this practice.

Finally, in the last 15 years a number of patients with recent-onset disease have died in intensive care units and permission has been given to remove organs for transplantation. The pancreas has thus been removed immediately after death, there has been no shortage of tissue and a full range of tests could be done (Dotta et al 2007).

Insulits

If the pancreas of a patient who has had type 1 diabetes for more than five years is studied, the great majority of islets will be seen to be insulin deficient. They consist of a normal number of the other hormone-secreting cells found in the islets of the pancreas (pancreatic polypeptide-secreting PP cells, glucagon-secreting A cells and somatostatin secreting D cells) (Foulis & Stewart 1984). There has thus been selective loss of the β cells. If the pancreas is studied at or within a year or two of clinical diagnosis, three types of islet are found (Gepts 1965, Foulis & Stewart 1984). Firstly, approximately 70% of the islets are insulin deficient (identical to those found in patients with prolonged disease). Secondly, there are islets containing β cells that are affected by insulitis (a chronic inflammatory infiltrate within the islet, Fig. 1) and, thirdly, there are insulin-containing islets which appear essentially normal. The finding that 18%

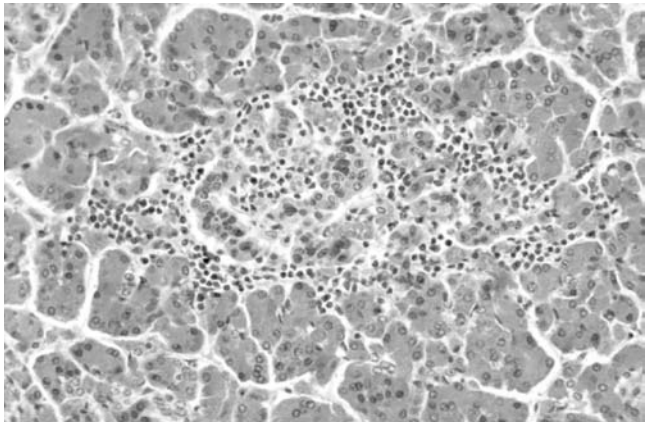


FIG. 1. Insulitis. There is a predominantly lymphocytic infiltrate in this islet.

of insulin containing islets but only 1% of insulin-deficient islets were affected by insulitis helped support the concept of there being an immunologically mediated destruction of β cells in the pathogenesis of type 1 diabetes (Foulis & Stewart 1984).

It can be seen therefore that within a given pancreas at clinical presentation there are islets where the β cells have been destroyed (insulin deficient), islets where the β cells are being destroyed (insulitis) and islets where the β cells are yet to be destroyed (normal). It has been argued that the pancreas in type 1 diabetes at clinical presentation is very similar qualitatively to the pancreas a few years after clinical presentation and also to the pancreas before clinical presentation. All three types of islet described above are present but the proportion of the islet types varies greatly with duration of the disease (Foulis 1989). Insulits affecting insulin containing islets has been observed six years after clinical presentation and in this pancreas 95% of islets were insulin deficient (Foulis et al 1986) By contrast in a pre-diabetic pancreas only 4% of islets were insulin deficient but insulitis was also observed (Foulis et al 1986). Thus it seems that the disease process in the pancreas is remarkably similar over a long period of time, with clinical presentation occurring when two thirds of the islets are insulin deficient (Foulis et al 1986). It follows that study of disease phenomena in the pancreas at clinical presentation should help to elucidate the pathogenesis of type 1 diabetes both at clinical presentation and in the pre-diabetic period.

Inflammatory cells in insulitis

Bottazzo et al (1985), in their case report, were the first to study the nature of the inflammatory infiltrate in insulitis. It consisted essentially of lymphocytes, with macrophages being inconspicuous. The majority of the lymphocytes were cytotoxic T cells. All studies on autopsy pancreases have repeated the observation that macrophages represent a minor population of the infiltrate. In a study of 87 affected islets from 12 autopsy pancreases the ratio of lymphocytes to macrophages was 10:1 and the average number of lymphocytes per inflamed islet was 85 (Foulis et al 1991). The first study of pancreatic biopsies reported no evidence of insulitis even in the four pancreases with residual β cells (Hanafusa et al 1990). Subsequent studies from the Osaka group however did report insulitis. Interestingly, their definition of insulits in the later studies was an islet infiltrated by two or more inflammatory cells (Itoh et al 1993) Even in this minimal (significant?) form of inflammation the predominant inflammatory cell was the $CD8^+$ T cell. These findings are consistent with destruction of β cells by cell-mediated cytotoxic T cell attack and do not support a major role for bystander damage by cytokines released by macrophages.

Fas and Fas ligand expression

Two groups have looked at Fas and Fas ligand (Fas-L) expression in insulitis. Fas-positive endocrine cells were detected in islets affected by insulitis but not in non-inflamed islets in diabetics or in normal control pancreatic islets (Stassi et al 1997, Moriwaki et al 1999). Interestingly, Moriwaki et al (1999) showed that while most B cells were Fas positive a significant minority of A cells also expressed this receptor. Infiltrating lymphocytes were Fas-L positive while islet endocrine cells were Fas-L negative. These observations have led to the hypothesis that cytokines such as interferon (IFN)γ, tumour necrosis factor (TNF)α or interleukin (IL)1, which induce Fas expression by islet endocrine cells *in vitro*, could be released in the insulitis process and cause the same effect *in vivo*. In this manner Fas-L-positive infiltrating cells in the inflamed islets could destroy Fas-positive β cells.

β cell apoptosis

A number of groups have looked for evidence of β cell apoptosis. No affected β cells were seen in pancreatic biopsies by the Osaka group (Moriwaki et al 1999), while others found evidence for plentiful apoptosis in β cells in autopsy pancreases using the TUNEL method (Meier et al 2005, Stassi et al 1997). In view of the lack of evidence of β cell regeneration one has to view an apoptosis prevalence of 6% of β cells (Meier et al 2005) as being extremely unlikely given the fleeting nature of apoptotic bodies and the very long time over which β cell destruction appears to take place clinically.

Aberrant expression of class II MHC by β cells

It was hypothesized that aberrant expression of class II MHC by insulin-secreting β cells (Fig. 2) could lead to their presenting self antigens, with resulting autoimmunity (Bottazzo et al 1983). β cells do not normally express class II MHC but they did show this phenomenon in pancreases of 21 out of 23 cases of recent-onset diabetes (Foulis et al 1987a). In these cases aberrant expression of class II MHC was found in 12% of insulin-containing islets, and double stains showed that it was confined to β cells being not present in A, D or PP cells. The phenomenon has also been described in pancreatic biopsies of two Osaka patients (Imagawa et al 1996). Half the islets in which β cells expressed class II MHC had no evidence of inflammation, raising the possibility that in a given islet this abnormality preceded insulitis (Foulis et al 1987a). β cells expressing class II MHC were not seen in 95 control pancreases from patients with a variety of diseases including type 2 diabetes, graft versus host disease, chronic pancreatitis, cystic fibrosis and enteroviral pancreatitis (Foulis et al 1987a).

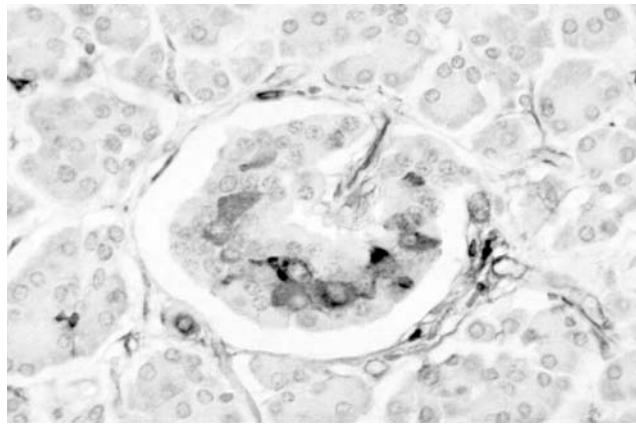


FIG. 2. Aberrant expression of class II MHC on endocrine cells. Double stains showed that these were β cells.

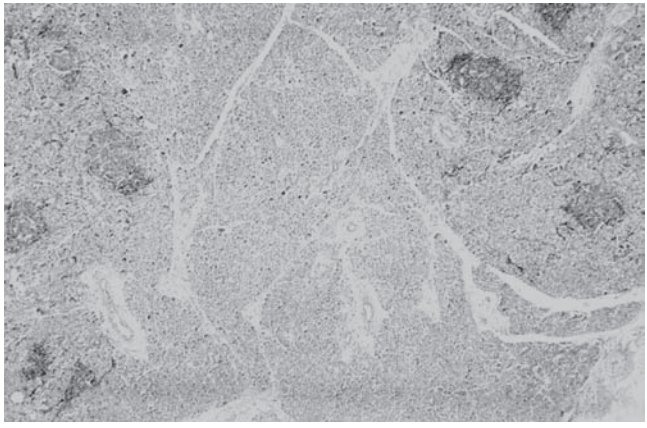
An antigen-presenting cell must express co-stimulatory molecules such as CD80 and CD86 as well as class II MHC to successfully present antigen to $CD4^+$ Th cells. Evidence against a pathogenetic role for aberrant expression of class II MHC on β cells has been the failure to demonstrate expression of either of these co-stimulatory molecules by β cells in pancreatic biopsies of patients with recent-onset type 1 diabetes (Imagawa et al 1996).

Hyperexpression of class I MHC by insulin-containing islets

Cytotoxic ($CD8^+$) T cells, which are the dominant cell type in insulitis, recognize antigen when it is presented in association with class I MHC by a target cell. Hyperexpression of class I MHC by the target cell is likely to enhance this engagement. A phenomenon unique to type 1 diabetes is hyperexpression of class I MHC by all the endocrine cells in insulin-containing islets (Foulis et al 1987a). 92% of insulin-containing islets hyperexpressed class I MHC in contrast to only 1% of insulin-deficient islets (Fig. 3). The phenomenon was not seen in islets in any of the 95 control pancreases in that study. Class I MHC hyperexpression of islet endocrine cells was induced *in vitro* by IFNα, IFNβ or IFNγ (Pujol-Borrell et al 1986). Forty per cent of the lymphocytes in the insulitis infiltrate expressed IFNγ

FIG. 3. (a) Islets in two lobules hyperexpress class I MHC. (b) This is a serial section to Fig 3a stained for insulin. Insulin-containing islets hyperexpress class I MHC in type 1 diabetes. (c) This serial section has been stained for glucagon. Numerous shrunken insulin deficient islets are present in the centre of the photograph which do not hyperexpress class I MHC.

(a)



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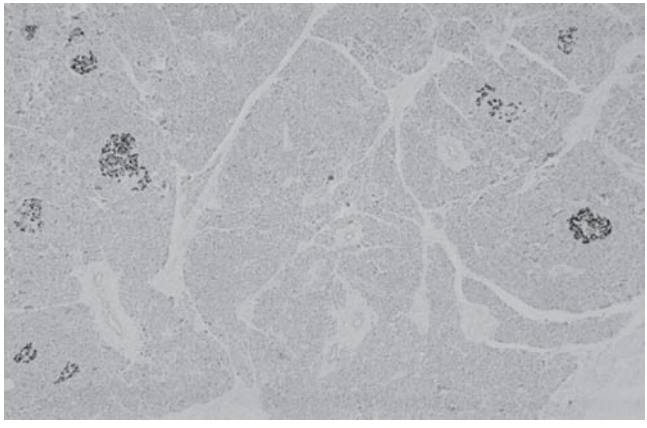

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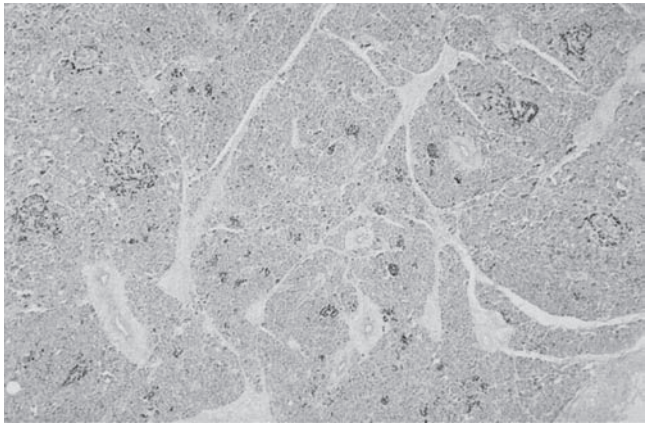

(Foulis et al 1991) so it might be supposed that this hyperexpression of class I MHC would be a secondary event following insulits. However, even when whole islets in multiple serial sections were studied it was clear that over half the insulin-containing islets which hyperexpressed class I MHC had no evidence of insulitis whatsoever. Thus it was argued that hyperexpression of class I MHC by insulin-containing islets was an earlier event in the disease process than insulitis. Comparison of class I hyperexpression and aberrant class II expression by β cells showed that all islets where the latter phenomenon was seen hyperexpressed class I MHC. By contrast 73% of islets which hyperexpressed class I MHC showed no evidence of aberrant expression of class II MHC on β cells. Thus hyperexpression of class I MHC also appeared to be an earlier event in the disease process within an islet than class II MHC expression by β cells. Finally it was noted that A and D cells hyperexpressed class I MHC when they lay adjacent to β cells in insulin-containing islets of type 1 diabetes patients, but not when they were physically divorced from β cells in insulin-deficient islets. This raised the possibility that the β cells were releasing a type 1 interferon that was causing the hyperexpression through a paracrine effect (Foulis et al 1987a).

β cells express IFNα in type 1 diabetes

An immunohistochemical analysis of IFNα expression in type 1 diabetes was therefore undertaken. β cells, but not A, D or PP cells expressed IFNα in all 28 pancreases from patients with recent onset type 1 diabetes. This expression was closely related to class I MHC hyperexpression. β cells expressing IFNα were found in 94% of islets which hyperexpressed class I MHC but only in 0.2% of islets which did not hyperexpress this complex. Among 80 control pancreases, β cells expressed significant IFNα in four cases of Coxsackie B viral pancreatitis but not in other pancreatic diseases (Foulis et al 1987b).

Possible sequence of immunological events in islets (Fig. 4)

The conclusion of the studies outlined above is that the first abnormality in an islet in type 1 diabetes is expression of IFNα by β cells. Secretion of this cytokine is likely to cause hyperexpression of class I MHC by all the endocrine cells within that islet. Aberrant expression of class II MHC is a later event, which probably occurs in a minority of islets, but it too appears to precede insulits. The finding that β cells secreted IFNα in enteroviral pancreatitis as well as type 1 diabetes raises the possibility that a non cytopathic viral infection of β cells is the initiating event in the disease process leading to autoimmune destruction of β cells and type 1 diabetes (Foulis 1989).