

Aberrant expression of Class II major histocompatibility complex molecules by B cells and hyperexpression of Class I major histocompatibility complex molecules by insulin containing islets in Type 1 (insulin-dependent) diabetes mellitus

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Summary. Twenty-three patients with recent onset Type 1 (insulin-dependent) diabetes in whom residual insulin secreting B cells were present and 12 patients with disease of more prolonged duration (maximum 9 years), 8 of whom had residual B cells, were studied. Aberrant expression of Class II major histocompatibility complex molecules was demonstrated immunohistochemically on insulin secreting B cells in 21 out of 23 patients with recent onset disease and 6 of the patients with more prolonged disease. No such expression was seen on glucagon secreting A cells or somatostatin secreting D cells. Islets where there was marked hyperexpression of Class I major histocompatibility complex molecules on islet endocrine cells were seen in all cases in which residual B cells were present. Ninety-two per cent of insulin containing islets but only 1% of insulin deficient islets exhibited

this phenomenon ($p < 0.001$, Chi-squared test). There was evidence to suggest that both these abnormalities of major histocompatibility complex expression preceded insulinitis within a given islet. They also appeared to be unique to Type 1 diabetes, being absent in pancreases of patients with Type 2 (non-insulin-dependent) diabetes, chronic pancreatitis, cystic fibrosis, graft-versus-host disease and Coxsackie B viral pancreatitis. The development of autoimmunity to B cells in Type 1 diabetes may be a “multistep” process in which abnormalities of major histocompatibility complex expression on islet endocrine cells are crucial events.

Key words: Pancreas, islets of Langerhans, antigen-immune response, autoimmunity, Type 1 diabetes mellitus, major histocompatibility complex.

Two factors are recognised to be involved in the pathogenesis of Type 1 (insulin-dependent) diabetes. Firstly, there is considerable evidence that the disease is an example of an organ-specific autoimmune disease in which the insulin secreting B cells are destroyed by an inflammatory cell infiltrate affecting the islets [1–3]. Secondly, there is a marked genetic component to the risk of acquiring the disease which is linked to Class II major histocompatibility complex (MHC) genes (HLA-DP - DQ and - DR in man). Thus, up to 98% of all patients with Type 1 diabetes possess either DR3 or DR4 genes [4].

It is thought that the principal function of Class II MHC products is to facilitate antigen presentation. T helper lymphocytes, which will initiate an immune response, only recognise the antigen to which they are directed if it is ‘presented’ to them by a cell expressing the same Class II MHC as themselves. It has been proposed that the initial event in many organ-specific autoimmune diseases may be aberrant expression of Class II MHC by the target cells [5]. If a cell did not normally express Class II MHC on its surface, then

any cell specific auto-antigens it may possess would never have been ‘presented’ to potentially autoreactive T helper lymphocytes. Thus, even though the latter may exist, there would be no immune response. If, however, the cell were induced to express Class II MHC, then it may become a functional antigen presenting cell. It may then “present” its particular cell surface antigen to potentially autoreactive T helper lymphocytes, which would then initiate an autoimmune response. This hypothesis is particularly attractive because it encompasses the agent linked to the genetic predisposition to Type 1 diabetes - DR type - with an event on the target cell, i. e. aberrant expression of Class II MHC.

Endocrine cells in the normal pancreas do not express Class II MHC [6, 7]. In the pancreas in Type 1 diabetes only the B cells are destroyed [3, 8]. Thus, if the hypothesis were valid in this disease, only the B cells would aberrantly express Class II MHC. Expression of Class II MHC products, confined to B cells, was initially reported in a single case of recent onset Type 1 diabetes where fresh frozen autopsy pancreas

was available [9]. Recently, with the advent of the monoclonal antibody TAL-1B5, which is capable of detecting a formalin resistant epitope of the alpha-chain of HLA-D molecules [10, 11], expression of Class II MHC on B cells was seen in pancreases from 12 patients with Type 1 diabetes [12]. All other endocrine cells were negative, and no aberrant expression was seen on B cells in Type 2 diabetes, cystic fibrosis or chronic pancreatitis [12].

A second abnormality of MHC expression has been observed in the pancreas in Type 1 diabetes. Bottazzo et al. [9] reported that there was marked hyperexpression of Class I MHC (HLA-A-B and -C in man) on endocrine cells in some islets. This phenomenon was not fully characterised, and in particular no mention was made of the types of endocrine cell affected.

The aims of the present report were to study these abnormalities of MHC expression in a large number of patients with Type 1 diabetes in an attempt to establish firstly, whether they were unique to the disease, and secondly, whether they were purely the result of inflammation within the islets or whether they occurred independently of the presence of insulinitis.

Subjects and methods

The pancreases of 35 patients who had died with Type 1 diabetes in whom the pancreatic histopathology was "classical" [3] were studied. This included all 14 cases which were reported previously [12]. Twelve patients had had diabetes for more than 1 year (maximum 9 years). Cases of prolonged duration diabetes were selected to include those in whom residual B cells were present. Twenty-three patients had died within a few weeks of onset of symptoms. Four of these patients had evidence of other autoimmune diseases ("polyendocrine diabetes"); two patients had thyrotoxicosis and two Addison's disease.

Pancreases from the following were also included as controls: (1) Thirty-eight autopsies on children who had died of a variety of conditions but in whom the pancreas was histologically normal. (2) Twenty patients who had died after receiving total body irradiation and bone marrow grafting as therapy for leukaemia (fifteen of these patients died with significant graft-versus-host disease). (3) Five pancreatic resections for chronic pancreatitis. (4) Autopsies on 10 children who had died of cystic fibrosis. (5) Autopsies on 10 patients who had Type 2 (non-insulin-dependent) diabetes. (6) Autopsies on 12 neonates who had died of culture proven Coxsackie B viral myocarditis.

All the tissues studied had been fixed in either buffered formalin or formol saline with or without added mercuric chloride. Four micron serial sections were cut from the paraffin embedded blocks of the pancreases from patients with Type 1 diabetes, those with Coxsackie B virus infection and those who had had marrow transplants. Adjacent serial sections from these cases, and three nonserial sections from all others, were stained with an indirect immunoperoxidase technique in which diaminobenzidine was the substrate, using the following primary polyclonal antisera: rabbit anti-Class I MHC heavy chain ("AI", gift from J.J. Neefjes, Amsterdam, The Netherlands), guinea pig anti-insulin (Wellcome, Dartford, UK) and rabbit anti-Class II MHC ("AII", gift from J.J. Neefjes). A minority of the Type 1 diabetic pancreases were stained using the same techniques with two other primary polyclonal antisera: rabbit anti-beta-2-microglobulin (Dako, High Wycombe, UK) and a second rabbit anti-Class II MHC ("UUII", gift from L. Klareskog, Uppsala, Sweden).

These latter two antisera were tested to confirm the findings using the antisera AI and AII. The following bridges for the indirect techniques were used: peroxidase conjugated rabbit anti-guinea pig, swine anti-rabbit and rabbit anti-mouse immunoglobulins (all Dako). In addition to the sections described, one pancreatic block from each of 3 cases [Case 8 (total number of blocks 3), Case 12 (total number of blocks 6) and Case 19 (total number of blocks 4); Table 1] was selected and 40 4- μ m serial sections cut. The middle two sections of these series were stained for Class I MHC (using antiserum AI) and insulin respectively. The remaining sections were all stained for Class II MHC (using antiserum AII). Insulin containing islets were identified on the section stained for insulin and the presence or absence of hyperexpression of Class I MHC and aberrant expression of Class II MHC noted by viewing each islet on the remaining serial sections. Over 90% of the insulin containing islets identified were assessed in each of these cases.

Detailed studies of the specificity of these various antisera have been published. The rabbit antiserum to Class I MHC, AI, was prepared against a mixture of purified heavy chains of HLA-B7 and HLA-B40 (13). In an immunoblot analysis of white cells from 21 different HLA blood type donors this antiserum reacted with all the HLA-B locus specificities tested, in addition to a number of A-locus products. An indirect way of looking at Class I MHC expression in tissue is to study the distribution of beta-2-microglobulin. This protein, which is coded for by a gene on a different chromosome from that which codes for Class I MHC, associates non-covalently with Class I MHC in the plasma membrane [14]. Thus, in addition to studying Class I MHC expression in the diabetic pancreases, the distribution of beta-2-microglobulin was also examined in an attempt to confirm the results. The distribution of immunoperoxidase staining on formalin fixed pancreas and tonsil using the antiserum AI and the antiserum to beta-2-microglobulin as primary antisera was compared to staining on fresh frozen pancreas and tonsil using the same antisera, and also the mouse monoclonal antibody W6/32 (Sera-Lab, Sussex, UK) which recognises all HLA-A and -B antigens [15]. The staining patterns were identical.

The rabbit antiserum, AII, was raised against the purified human Class II antigens, DR and DQ [16]. Part of the antigen had been denatured by heat. The serum recognised human Class II alpha chains and a number of Class II beta chains in immunoblot analysis, and it reacted with DP, DQ and DR. The specificity of the second anti-Class II MHC antiserum (UUII) has also been previously described in detail [17]. The staining patterns using immunohistochemistry on formalin fixed pancreas and lymph node with these two antisera, and the mouse monoclonal antibody TAL-1B5 (directed against HLA-D region alpha chains [10] were compared with their staining on fresh frozen pancreas and lymph node. The staining was identical in all cases.

All the rabbit antisera to MHC molecules and beta-2-microglobulin performed satisfactorily with the immunoperoxidase techniques on tissue fixed in any of the commonly used fixatives. When normal rabbit serum was substituted for these antisera in an indirect immunoperoxidase technique, no staining of any description was seen on either diabetic or normal pancreases.

Double staining techniques were developed to show which hormone producing cells expressed Class II MHC or hyperexpressed Class I MHC. Following an overnight incubation at 4°C with either AI or AII, the sections were incubated with peroxidase conjugated swine anti-rabbit immunoglobulins for 30 min at room temperature. The reaction was developed using diaminobenzidine as substrate. Double staining for insulin was done using a subsequent 4-h incubation of the guinea pig anti-insulin anti-serum followed by a 30-min incubation in rabbit anti-guinea pig immunoglobulins (Dako). Finally, there was a 30 min incubation with fluorescein conjugated donkey anti-rabbit immunoglobulins (Scottish Antibody Production Unit, Carlisle, UK). Repeated washings were done between steps using tris buffered saline. Double staining for Class I and II MHC and other pancreatic hormones utilised the same method up to the development with diaminobenzidine. This was followed by a 15-min incubation in normal swine serum, a 4-h incubation with either rabbit anti-glucagon (Guildhay, Guildford, UK) or rabbit anti-somatostatin

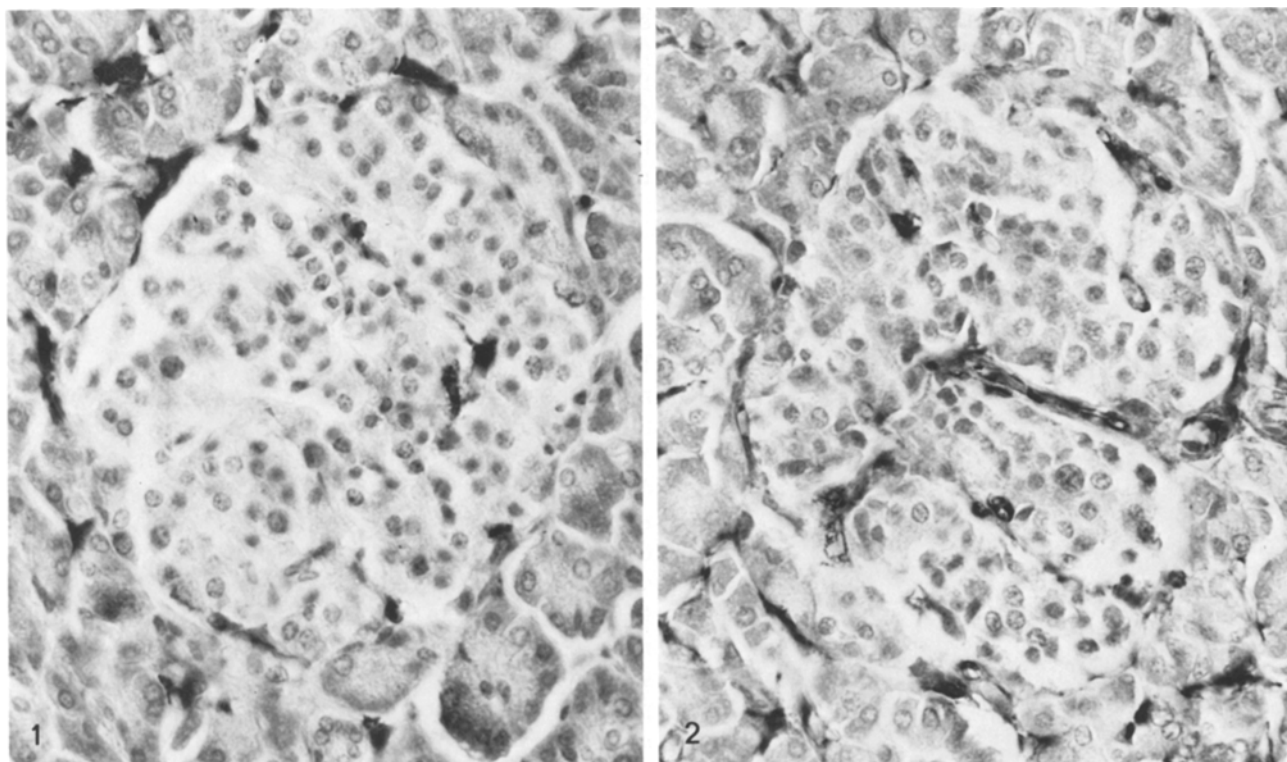


Fig. 1. Class II MHC expression in normal autopsy pancreas. Only the endothelial cells are positive. (Indirect immunoperoxidase using anti-serum AII $\times 500$)

Fig. 2. Class I MHC expression in normal autopsy pancreas. This is an adjacent section to that photographed in Fig. 1. Staining is again seen on endothelial cells. Class I MHC is almost undetectable on endocrine cells using this technique. (Indirect immunoperoxidase using antiserum AI $\times 500$)

(RIA UK Ltd., Tyne & Wear, UK) and finally a 30-min incubation with fluorescein conjugated donkey anti-rabbit immunoglobulins. Again, repeated washings with tris buffered saline were done in between incubations. Double-stained sections were viewed using a Leitz Orthomat microscope. Control pancreases and spleen were tested on all occasions and there were no cross reactions observed between the two steps of these double techniques.

Statistical analysis

The proportion of insulin containing islets which hyperexpressed Class I MHC was compared to the proportion of insulin deficient islets which exhibited this phenomenon. Statistical significance was assessed using the Chi-squared test.

Results

Normal autopsy pancreas

The distribution of Class II MHC in the normal autopsy pancreas, as assessed by the antiserum AII, was the same as that described previously using the antibody TAL-1B5 (Fig. 1) [12]. Thus, in the islets only the endothelial cells were stained, while in exocrine tissue occasional ducts were positive but acini were generally negative. There was almost undetectable staining for Class I MHC antigens on islet endocrine cells using

the antiserum AI. Endothelial cells and nerve fibres were strongly positive, but the exocrine tissues stained relatively weakly (Fig. 2).

The pancreas in Type 1 diabetes

Four principal variables were analysed: (1) insulin content of islets, (2) presence or absence of insulinitis, (3) presence or absence of aberrant expression of Class II MHC on islet endocrine cells, (4) degree of expression of Class I MHC antigens on islet endocrine cells. Tables 1 and 2 give the results of the analysis of islet staining on 3 consecutive serial sections from each case stained for Class I MHC, insulin and Class II MHC respectively. The degree of expression of Class I MHC on islet endocrine cells and the presence or absence of insulin in an islet could be assessed fairly confidently on single sections of tissue stained for the respective antigens. However, aberrant Class II MHC expression on endocrine cells and the presence of an inflammatory infiltrate could be quite focal within an islet, and the presence of these was liable to be underestimated if only one section from each case was examined. Thus, three cases [8, 12, 19] were chosen for more detailed study on the basis that blocks were available and that the phenomena under investigation were present. For-

ty serial sections were cut from one of the several pancreatic blocks available for each case and the middle two sections stained for Class I MHC products and in-

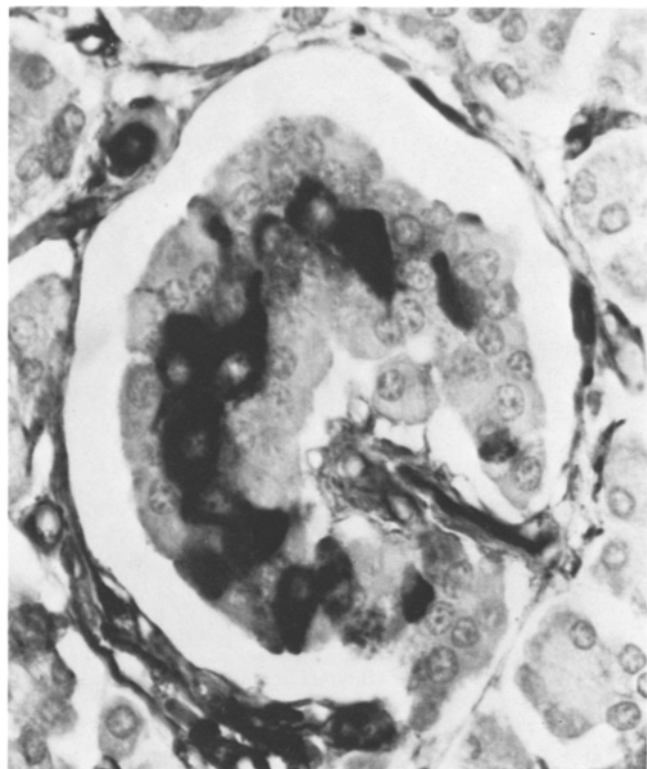


Fig. 3. Class II MHC expression on islet endocrine cells in Type 1 diabetes. The positive cells show uniform cytoplasmic staining and lie flanked by negatively staining endocrine cells. An adjacent section showed the presence of B cells in this islet. (Intraperitoneally using antiserum AII $\times 800$)

sulin respectively. The remaining sections were stained for Class II MHC products. From this, the presence of focal insulinitis, or infrequent aberrant expression of Class II MHC on endocrine cells, could be assessed for any islet identified on the middle two sections. The results of this analysis are given in Table 3.

Aberrant expression of Class II MHC products on islet endocrine cells

Insulin containing islets were present in all 23 cases of recent onset diabetes and in 8 out of 12 cases where the disease had been present for more than one year. Aberrant expression of Class II MHC on endocrine cells was seen in 21 of the recent onset cases and 6 of the prolonged duration cases (Fig. 3). The maximum duration of diabetes where this phenomenon was observed was 9 years. Endocrine cells expressing Class II MHC were seen in 377 out of 2744 insulin containing islets but not in 6842 insulin deficient islets. Double stains for hormones and Class II MHC confirmed the previous observation that only B cells expressed this antigen (Figs. 4, 5). When 2 adjacent serial sections were stained with AII and UUII, both polyclonal antisera to Class II MHC, the results were identical. As with the previous study using TAL-1B5 [12], the staining for Class II MHC was cytoplasmic (Fig. 3).

It is recognised that the detailed results of the cases reported previously [12, Table 1] were not identical to those observed in this study. This was because fresh serial sections were cut for the present study.

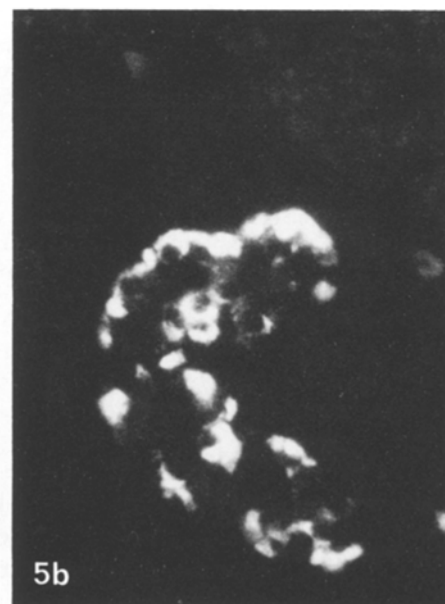
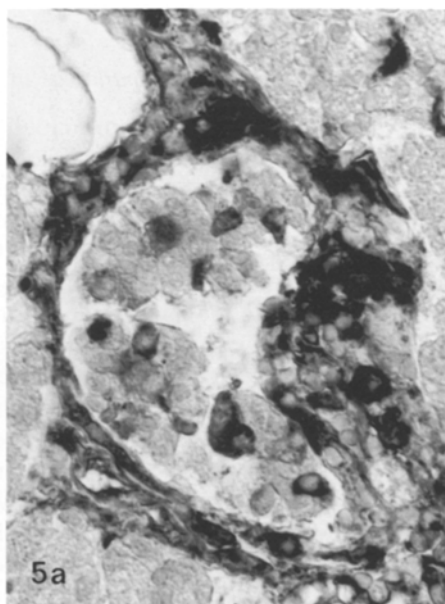
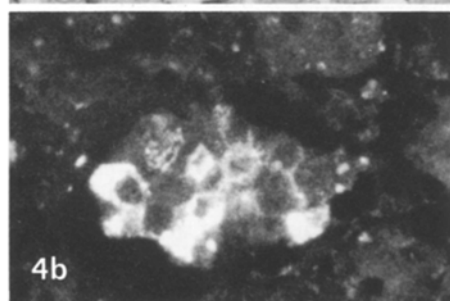
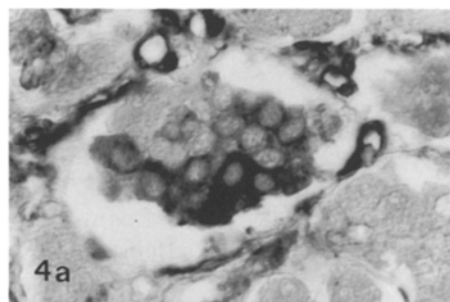


Fig. 4a and b. The section has been double stained by an immunoperoxidase technique for Class II MHC (a) and by an indirect immunofluorescence technique for insulin (b). Several B cells are present which aberrantly express Class II MHC ($\times 500$)

Fig. 5a and b. The section has been double stained by an immunoperoxidase technique for Class II MHC (a) and by an indirect immunofluorescence technique for glucagon (b). Class II MHC positive endocrine cells do not contain glucagon ($\times 500$)

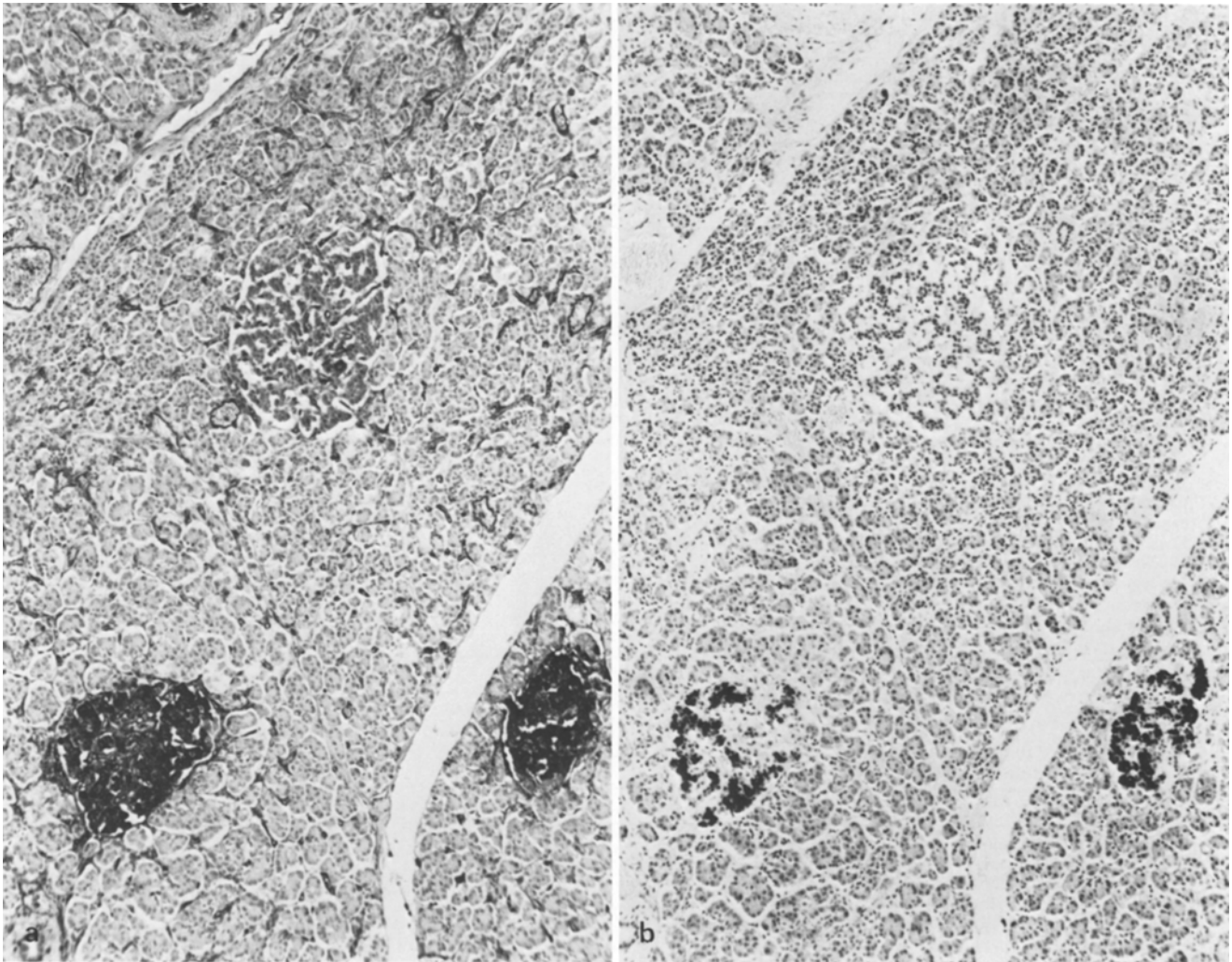


Fig. 6 **a** There is marked hyperexpression of Class I MHC on the endocrine cells in the two islets at the bottom of the picture. By contrast, the islet at the top of the picture shows markedly diminished expression of this product. **b** The serial section has been stained for insulin and shows that the islet at the top of the picture is insulin deficient ($\times 70$)

Degree of Class I MHC expression and insulin content of islets

A striking observation was the very marked degree of hyperexpression of Class I MHC on insulin containing islets (Fig. 6). In islets where this was present, there was not only increased cytoplasmic staining but also considerable accentuation in the region of the plasma membrane of cells. Class I MHC hyperexpression was present in all 31 patients with residual insulin containing islets. Furthermore, it was found in 2517 out of 2744 such islets (92%) but was only seen in 82 out of 6842 insulin deficient islets (1%, $p < 0.001$ Chi-squared test) (Table 1). The pattern of staining when two adjacent serial sections were stained with the antisera directed against Class I MHC heavy chain (AI) and beta-2-microglobulin, respectively, was identical. In contrast to the findings with Class II MHC expression, all the endocrine cells in a given insulin containing islet appeared to hyperexpress Class I MHC. This was confirmed by double stains for Class I MHC and the re-

spective hormones (Figs. 7, 8). Thus, it appeared that when glucagon secreting A cells and somatostatin secreting D cells were adjacent to B cells in an insulin containing islet in this disease they hyperexpressed Class I MHC, but when they were physically divorced from B cells, in an insulin deficient islet, they ceased to hyperexpress Class I MHC. (The findings with PP cells were not formally assessed, since all the insulin containing islets examined were in the glucagon-rich lobe).

Occasional insulin containing islets in which there was no hyperexpression of Class I MHC were observed in some cases. It was interesting to note that these islets tended to be grouped together in a non-random manner, often within the same pancreatic lobule (Fig. 9).

The relevance of the serial section study of "whole" islets

Comparison between the findings for the 3 cases 8, 12 and 19 given in Tables 1 and 3 shows that when 40 serial sections were not done the frequency of insulinitis

Table 1. Insulin content of islets, insulinitis, hyperexpression of Class I MHC and aberrant expression of Class II MHC on islet endocrine cells in Type 1 diabetes: detail of cases

Case	Age	Sex	Duration of diabetes	Insulin containing islets (ICI)				Insulin deficient islets (IDI)				Islets with Class II MHC positive endocrine cells	
				Total	No. with insulinitis	No. with hyperexpression of Class I MHC	No. with aberrant expression of Class II MHC on endocrine cells	Total	No. with insulinitis	No. with hyperexpression of Class I MHC	No. with aberrant expression of Class II MHC on endocrine cells	Insulinitis present	Insulinitis absent
Recent onset diabetes													
1	18 months	F	1 wk	116	112	115	9	1239	8	6	0	9	0
2	21 months	F	2 wks	16	11	16	2	69	1	2	0	2	0
3	2 years	M	2 wks	19	1	16	0	177	3	7	0	0	0
4	3 years	F	6 wks	88	30	88	4	43	1	3	0	0	4
5	3 years	M	4 days	59	17	59	5	292	1	0	0	4	1
6	3 years	M	3 mths	45	6	45	16	100	2	0	0	4	12
7	5 years	F	12 wks	29	2	27	1	21	0	0	0	1	0
8	6 years	M	6 days	128	35	128	22	359	6	4	0	20	2
9	6 years	F	few wks	36	4	36	4	79	1	0	0	1	3
10	7 years	M	3 days	37	19	37	7	148	6	4	0	7	0
11	8 years	M	few mths	118	2	79	8	69	1	1	0	1	7
12	10 years	M	3 wks	117	0	111	15	150	0	3	0	0	15
13	11 years	M	2 wks	29	0	29	5	13	0	2	0	0	5
14	12 years	F	3 wks	36	5	36	1	37	6	0	0	0	1
15	14 years	F	few wks	135	61	134	10	353	7	1	0	10	0
16	16 years	M	few wks	18	4	16	4	29	0	1	0	4	0
17	17 years	M	2 days	377	226	369	28	211	6	2	0	23	5
18	17 years	M	1 wk	358	117	357	84	217	28	36	0	40	44
19	21 years	M	3 wks	329	35	325	113	278	1	10	0	24	89
Recent onset polyendocrine diabetes													
20	8 years	F	3 weeks	114	5	80	0	99	0	0	0	0	0
21	13 years	F	2 months	101	5	101	1	26	0	0	0	0	1
22	18 years	F	few weeks	185	7	140	8	18	0	0	0	0	8
23	18 years	M	few weeks	61	4	54	4	35	0	0	0	1	3
Prolonged duration diabetes													
24	5 years	F	15 months	3	0	3	0	142	0	0	0	0	0
25	15 years	F	2 years	73	0	45	4	63	0	0	0	0	4
26	3 years	F	2 years	0	1	0	0	278	0	0	0	0	0
27	9 years	F	2 years	3	0	3	2	26	0	0	0	1	1
28	10 years	F	2 years	0	0	0	0	50	0	0	0	0	0
29	5 years	M	2 years	0	0	0	0	252	0	0	0	0	0
30	16 years	F	4 years	12	0	8	2	142	0	0	0	0	2
31	8 years	F	5 years	12	6	12	10	550	1	0	0	5	5
32	11 years	M	6 years	32	1	32	6	342	0	0	0	0	6
33	18 years	F	6 years	23	0	2	0	306	0	0	0	0	0
34	20 years	M	6 years	0	0	0	0	449	0	0	0	0	0
35	18 years	F	9 years	35	0	14	2	180	0	0	0	0	2
Total				2744	716	2517	377	6842	79	82	0	157	220
				26% of ICI 92% of ICI 14% of ICI				1% of IDI 1% of IDI					

and aberrant expression of Class II MHC on islets was seriously underestimated. Thus, when only a single section of each block was stained in these 3 cases 12% of insulin containing islets were affected by insulinitis and insulinitis was not seen in case 12 (Table 1). The "whole" islet study of a single representative block of each case showed that 44% of insulin containing islets were inflamed and insulinitis was in fact present in case 12. Similarly, in Table 1 26% of insulin containing islets from these cases were seen to contain B cells expressing class II MHC, but this figure rose to 67% when "whole" islets were examined. However, comparison between the results in Table 1 and Table 3 shows that the frequency of hyperexpression of Class I MHC by insulin containing islets could be accurately estimated from a single section. The explanation for the disparity is that insulinitis and aberrant expression of

Class II MHC can be quite focal within an islet and that maximum accuracy of the frequency of these phenomena in Type 1 diabetes can only be obtained by study of "whole" islets.

The relationship between Class I MHC hyperexpression and aberrant expression of Class II MHC

Table 3 shows that insulin containing islets could be found which did not hyperexpress Class I MHC. These islets showed no evidence of aberrant expression of Class II MHC. It also shows that 27% of the islets where there was hyperexpression of Class I MHC had no evidence of Class II MHC expression on B cells. The remaining islets exhibited both phenomena. Thus, no islets were seen where the only abnormality of

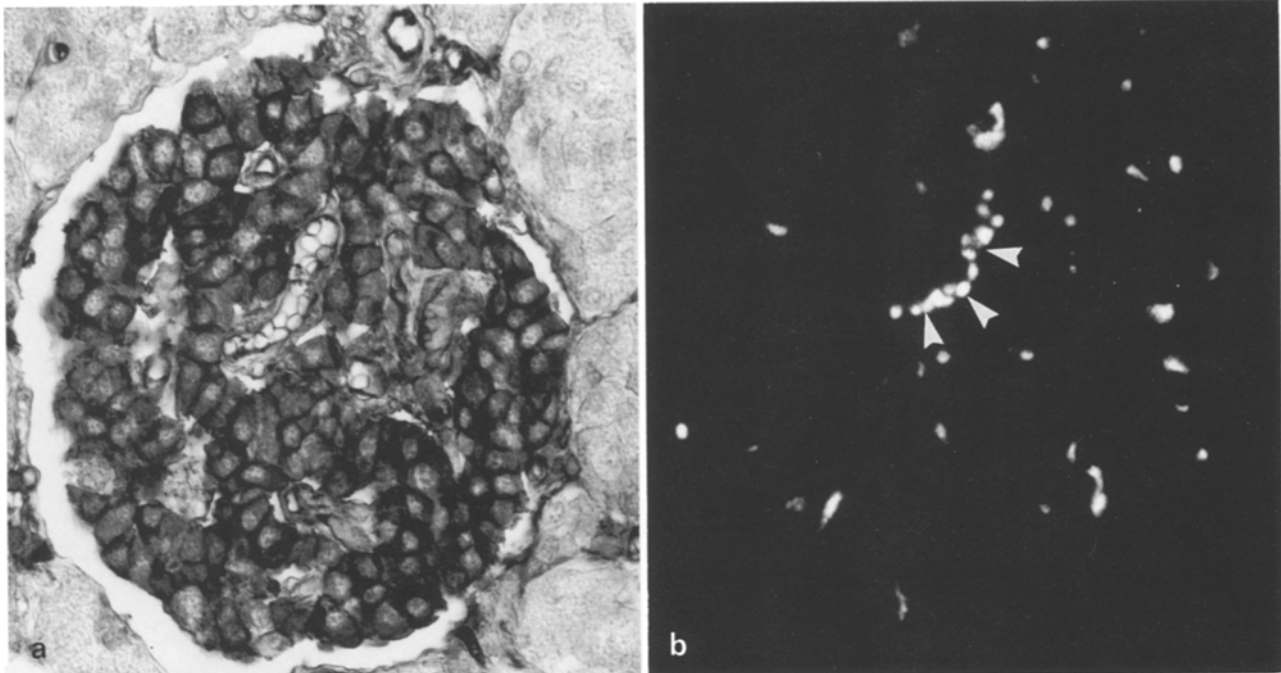


Fig. 7. The section has been double stained by an indirect immunoperoxidase technique for Class I MHC (a) and by an indirect immunofluorescence technique for insulin (b). Note the relatively low number of B cells compared to the number of endocrine cells which are hyperexpressing Class I MHC. Arrow heads mark autofluorescing erythrocytes ($\times 500$)

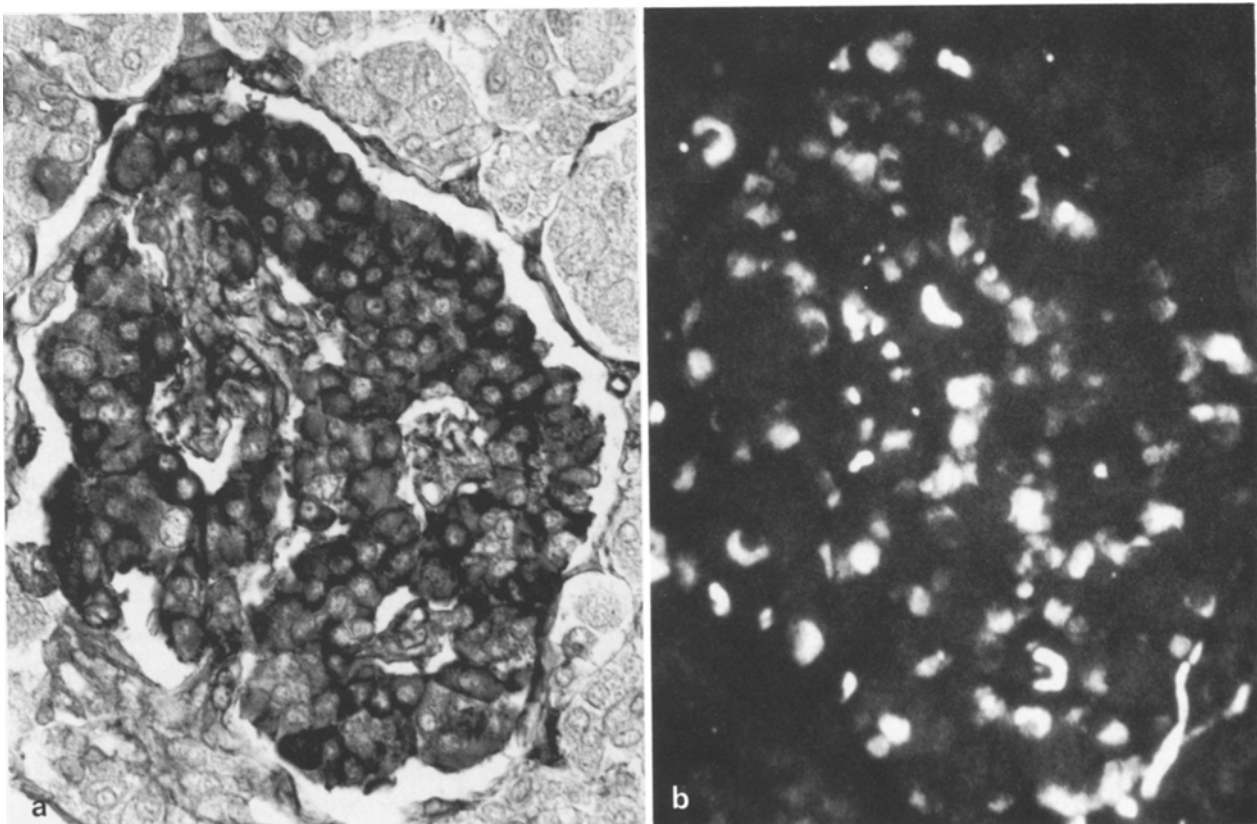


Fig. 8. The section has been double stained by an indirect immunoperoxidase technique for Class I MHC (a) and by an indirect immunofluorescence technique for glucagon (b). A cells hyperexpress Class I MHC in this islet ($\times 500$)

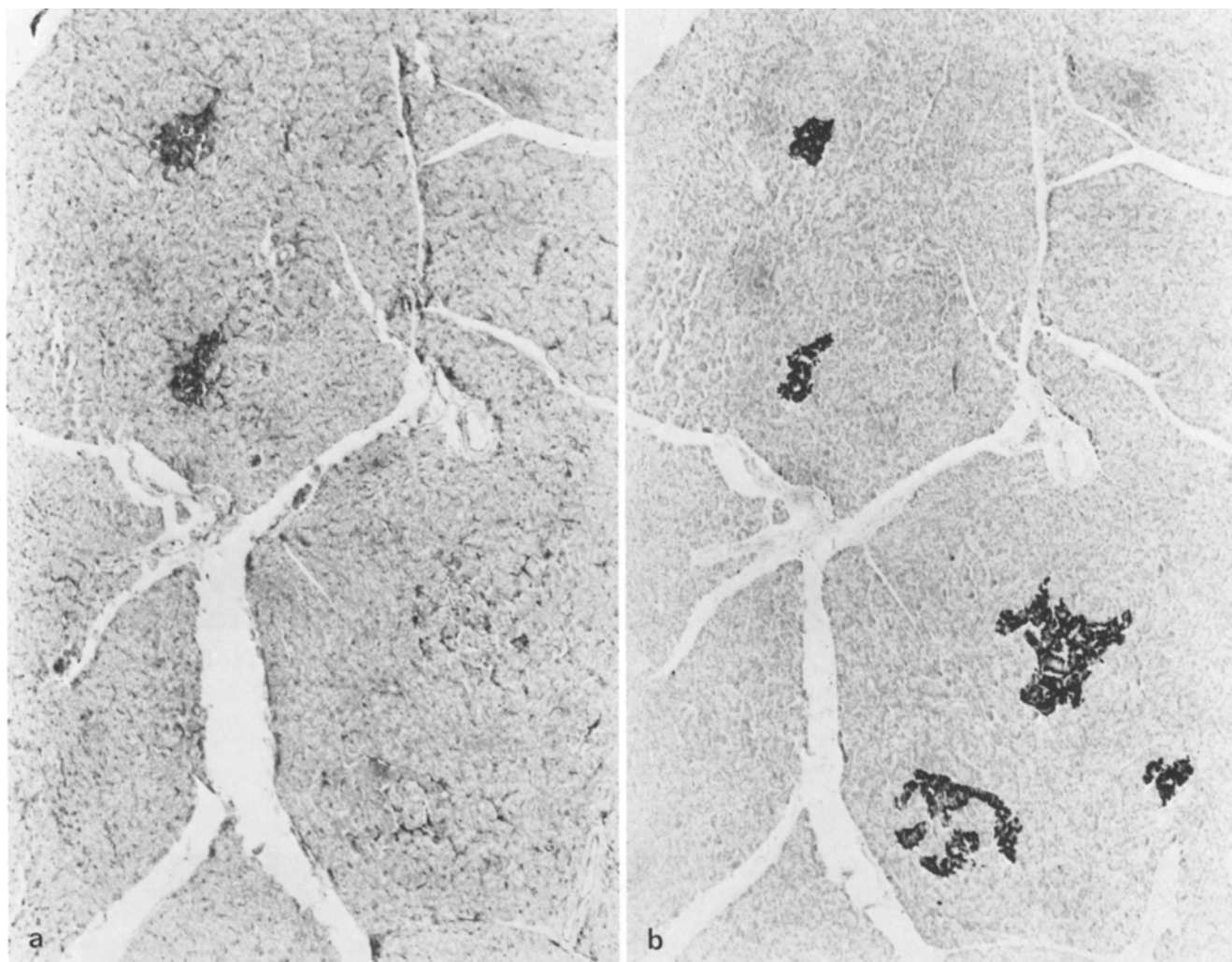


Fig. 9 **a** There is hyperexpression of Class I MHC by two islets in the top left of the picture. [Indirect immunoperoxidase using antiserum AI ($\times 500$)]. **b** The serial section shows insulin containing islets in the pancreatic lobule in the bottom right of the picture which do not hyperexpress Class I MHC [indirect immunoperoxidase for insulin ($\times 500$)]

MHC expression was the presence of Class II molecules on B cells.

The relationship between insulinitis and abnormalities of MHC expression

All insulin containing islets affected by insulinitis hyperexpressed Class I MHC. Interestingly, such hyperexpression was not necessarily found in inflamed insulin deficient islets. However, many of the islets in which hyperexpression of Class I MHC was observed were not affected by insulinitis (Table 3).

In the serial section study of "whole" islets half of the islets in which aberrant expression of Class II MHC was seen had no evidence of insulinitis. In case 8 (Table 3) it was noted that insulin containing islets could be found where there was insulinitis but no evidence of Class II MHC expression on endocrine cells.

Polyendocrine diabetes

While there were too few cases to draw firm conclusions, the results in Table 2 suggest that when patients with recent-onset polyendocrine diabetes were compared to the remainder of the patients with Type 1 diabetes of short duration not only were fewer insulin containing islets inflamed, but also only 2.8% of insulin containing islets had aberrant Class II MHC expression compared to 16% for the others. Similarly, fewer insulin containing islets hyperexpressed Class I MHC in the recent onset polyendocrine diabetic patients.

Class I and II MHC expression in pancreatic diseases other than Type 1 diabetes

While there was significant pancreatic inflammation in 13 of the bone marrow grafted patients (two of whom

Table 2. Insulin content of islets, insulinitis, aberrant expression of Class II MHC and hyperexpression of Class I MHC on islet endocrine cells in Type 1 diabetes: summary of cases

Number of	Non polyendocrine recent onset diabetes	Polyendocrine recent onset diabetes	Prolonged duration diabetes (more than 1 year)
Cases	19	4	12
Cases with ICI	19	4	8
ICI	2090	461	193
IDI	3884	178	2780
Cases with insulinitis	17	4	3
Cases with aberrant expression of Class II MHC on ICI	18	3	6
ICI with aberrant expression of Class II MHC	338	13	26
IDI with aberrant expression of Class II MHC	0	0	0
ICI with insulinitis	687	21	8
Islets with aberrant expression of Class II MHC and insulinitis	150	1	6
Islets with aberrant expression of Class II MHC but no insulinitis	188	12	20
Cases with hyperexpression of Class I MHC on ICI	19	4	8
ICI which hyperexpress Class I MHC (97% of ICI)	2023	375	119
IDI which hyperexpress Class I MHC (2% of IDI)	82	0	0
Islets with aberrant expression of Class II MHC but no hyperexpression of Class I MHC	0	0	0

ICI=insulin containing islets; IDI=insulin deficient islets

had no clinical evidence of graft-versus-host disease), the inflammatory infiltrate was diffuse throughout the pancreas with no evidence of specific insulinitis or endocrine cell damage. Marked endocrine cell necrosis affecting A cells as well as B cells was seen in 4 of the 12 neonates who had died of culture proven Coxsackie B viral myocarditis. In 3 of these 4 there was a prominent insulinitis characterised by lymphocytes and eosinophils.

No evidence of aberrant expression of Class II MHC was seen on endocrine cells in the pancreases of the 20 bone marrow grafted patients, the five resections for chronic pancreatitis, the 10 cases of cystic fibrosis, the 10 cases of Type 2 diabetes and the 12 cases with Coxsackie B viral infection. In pancreases where inflammation was present there was a moderate level of

Table 3. Insulinitis, aberrant expression of Class II MHC and hyperexpression of Class I MHC on islet endocrine cells of insulin containing islets: results of study of 40 serial sections on 3 cases

Number of	Case 19	Case 8	Case 12
ICI counted	50	40	40
ICI with insulinitis	16	39	2
ICI with aberrant expression of Class II MHC	44	26	18
ICI with aberrant expression of Class II MHC and insulinitis	16	26	2
ICI with aberrant expression of Class II MHC and no insulinitis	28	0	16
ICI with hyperexpression of Class I MHC	48	40	32
ICI with hyperexpression of Class I MHC but no aberrant expression of Class II MHC	4	14	14
ICI with no hyperexpression of Class I MHC but presence of aberrant expression of Class II MHC	0	0	0
ICI with insulinitis and hyperexpression of Class I MHC	16	39	2

ICI=insulin containing islets

staining for Class II MHC on the exocrine pancreas. In the inflamed pancreases there was also considerable hyperexpression of Class I MHC diffusely throughout the pancreas. This was most marked in cases of cystic fibrosis. Usually, however, the degree of expression of Class I MHC on islets was less than that observed in exocrine tissue. In no disease did the degree of expression of Class I MHC on islets match that seen on insulin containing islets from patients with Type 1 diabetes.

Discussion

Evidence is presented here that aberrant expression of Class II MHC, confined to B cells, and hyperexpression of Class I MHC by all the endocrine cells in insulin containing islets are as pathognomonic of Type 1 diabetes as are the presence of insulinitis and loss of B cells.

Pancreatic diseases in which there was a marked chronic inflammatory cell infiltrate were chosen as controls because in them release of immune modulators known to affect pancreatic endocrine cell MHC expression might be expected to be present [18–20]. While considerable expression of Class I and II MHC products was seen on ducts and acini in these diseases the phenomena of MHC expression seen on endocrine cells in Type 1 diabetes were not present. Aberrant expression of Class II MHC has been observed in a number of organs in graft-versus-host disease [21, 22], but was absent in the endocrine pancreas in patients with this disease in the present study. Class II MHC expression on B cells in Type 1 diabetes is unlikely to

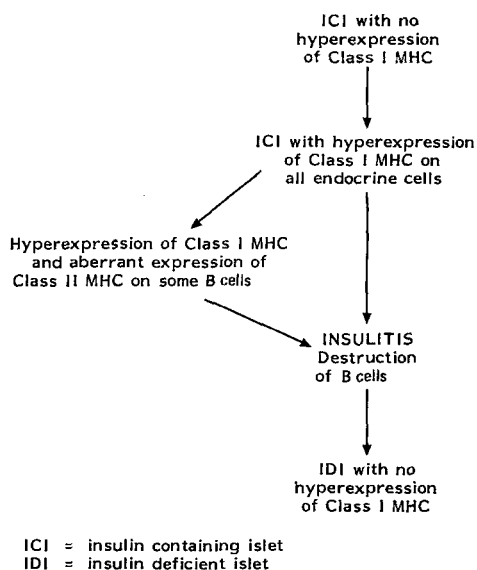


Fig. 10. Proposed sequence of events within individual islets in Type 1 diabetes

be the result of incipient death of these cells since such expression was not observed in the 4 cases of Coxsackie B viral pancreatitis where endocrine cell necrosis was present. Equally, the abnormalities of MHC expression are not thought to be the result of the metabolic derangements of diabetes since they were not seen in the pancreases of patients with Type 2 diabetes or of the two young patients with cystic fibrosis who were diabetic.

If it is accepted that insulinitis, destruction of B cells, expression of Class II MHC by B cells and hyperexpression of Class I MHC by insulin containing islets are the characteristic lesions of Type 1 diabetes, in what sequence do they occur within a given islet? It is recognised that any answers to this question are liable to be speculative since the earliest opportunity to observe these changes in the human pancreas is when approximately 70% of the B cells have been destroyed. However, there is merit in the argument that the pre-diabetic pancreas may be similar qualitatively to the pancreas at clinical presentation [12].

Based on the present material, there is evidence to suggest that within an individual islet hyperexpression of Class I MHC on all endocrine cells in an insulin containing islet precedes aberrant expression of Class II MHC on B cells. From Table 3 it can be seen that, while the majority of islets studied exhibited both abnormalities of MHC expression, islets were present where the only abnormality was hyperexpression of Class I MHC; however, no islets were seen where the only abnormality was aberrant expression of Class II MHC on B cells.

Similar arguments can be put forward to suggest that Class I MHC hyperexpression precedes insulinitis. Approximately half of the islets where this hyperexpression was seen had no evidence of insulinitis, but all

inflamed insulin containing islets hyperexpressed Class I MHC (Table 3). Equally, inflamed insulin deficient islets could be found in which there was no hyperexpression of Class I MHC on A and D cells.

It has previously been argued that within a given islet aberrant expression of Class II MHC on B cells precedes insulinitis [12]. In the present study of "whole" islets (Table 3) half of the 88 islets in which B cell Class II MHC expression was seen had no evidence of insulinitis. The absence of such expression on B cells in pancreatic inflammatory diseases and the failure of interferon-gamma and other inflammatory mediators to selectively induce Class II MHC expression on B cells but not other pancreatic endocrine cells *in vitro* [18–20] are in keeping with this conclusion.

The proposed sequence of events within an individual islet in Type 1 diabetes is therefore as follows (Fig. 10). The earliest demonstrable abnormality may be hyperexpression of Class I MHC. This may be followed either directly by insulinitis [inflamed islets could be found in Case 8 (Table 3) which did not have aberrant expression of Class II MHC] or by aberrant expression of Class II MHC on some B cells and then by insulinitis. B cells are killed in this inflammatory process, leaving an islet consisting of A, D and PP cells which no longer hyperexpress Class I MHC.

The BB rat has been used as a model of Type 1 diabetes and in this animal aberrant expression of Class II MHC by B cells is thought to be a late event which follows insulinitis within a given islet [23]. In keeping with this observation has been the demonstration that interferon-gamma caused isolated B cells from pre-diabetic BB/E rats to express Class II MHC *in vitro* [24]. B cells from normal Wistar rats did not exhibit this phenomenon in culture. This suggests that there may be a genetic abnormality of control of Class II MHC expression by B cells in these animals. A similar genetic abnormality of control of Class II MHC expression by B cells may be crucial in the pathogenesis of Type 1 diabetes in man. In the human pancreatic isograft study described by Sibley et al. [25], B cells genetically identical to those of the diabetic twins were destroyed within weeks of transplant by an islet infiltrate which included T lymphocytes. These inflammatory cells would be expected to be secreting interferon-gamma (immunosuppression was not given in 3 of the 4 patients studied). In spite of this, Class II MHC expression was not seen in B cells in the transplanted pancreases. This suggests that interferon-gamma may not be able to cause human B cells to express Class II MHC even when they are genetically identical to those of a patient with Type 1 diabetes.

If these abnormalities of islet MHC expression in Type 1 diabetes are not the result of islet inflammation, what is their cause? The intriguing aspect of the phenomenon of hyperexpression of Class I MHC in insulin containing islets was that A and D cells in these islets also hyperexpressed these molecules. One possible

explanation for this finding would be that the B cells in islets in which Class I hyperexpression was observed were secreting a substance which had a paracrine effect on the adjacent A and D cells, causing them also to hyperexpress Class I MHC. Candidates for this role might include interferon-alpha and -beta. Both these interferons have been shown to cause hyperexpression of Class I MHC on pancreatic endocrine cells in vitro [18]. A common stimulus for release of these substances from epithelial and connective tissue cells is viral infection. A B-cell specific, non-cytopathic viral infection could also be a possible candidate for causing aberrant Class II MHC expression by these cells in a genetically predisposed host. Recently it has been shown that rat astrocytes expressed Class II MHC products in vitro following non-infective interaction with a murine neurotropic coronavirus [26].

Induction of autoimmunity in Type 1 diabetes could be a "multistep" process in which the key event, but not necessarily the first event, is aberrant expression of Class II MHC by B cells.

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