Original articles

TRANSPLANT International

Transplant Int (1991) 4: 195–199

The immunology of the human foetal pancreas aged 8–13 gestational weeks

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Received March 4, 1991/Accepted May 23, 1991

Abstract. The expression of MHC class I and class II antigens by the human foetal pancreas (HFP) during the first trimester is poorly documented. Using immunohistochemical techniques, we analysed 37 HFPs aged 8–13 gestational weeks (gw) and compared the results with those of 9 HFPs aged 14–16 gw. In all of the specimens, the ductal cells were class I- and class II-negative. Islets and endothelial cells expressed class I but were class II$^-$. Interstitial class II$^+$ cells included macrophages, B lymphocytes and dendritic-like cells that were negative for macrophage markers. While the frequency of class II$^+$ cells in the HFP remained constant from 8 to 13 gw, a threefold increase was observed from the end of the 13th gw to the 16th gw. In conclusion, the lower density of interstitial class II$^+$ cells in HFPs aged 8–13 gw indicates that immunomodulation is likely to be more successful in this age group.

Key words: Pancreas, foetal, immunology – Foetal pancreas, immunology – Immunology, foetal pancreas

The rationale for using the foetal pancreas to treat diabetes mellitus is based on the premise that (1) it will continue to grow in its new milieu and (2) it confers immunological advantages compared to the adult pancreas.

Syngeneic transplantation of the foetal pancreas has been performed successfully in rodents. After a lag period during which islets differentiate from the immature ductal cells, the graft becomes functionally competent and reverses experimental diabetes mellitus [4, 18]. The age of the donor pancreas influences graft outcome [5, 16]. The optimal age varies between species. In humans, the evidence suggests that human foetal pancreases (HFPs) obtained during the first trimester have the greatest growth potential in vitro as well as in vivo [29, 30]. Clinical HFP transplantation has not been successful so far; however, one must bear in mind that the donor age has usually exceeded 13 gw in reported cases [14, 26]. The poorer proliferative capacity of such HFPs undoubtedly had an adverse effect on the fate of the grafts. It is also likely that both immunological and functional factors contributed to the eventual fate of these allografts, although firm histological evidence is lacking [26].

HFPs older than 13 gw contain class II$^+$ immunostimulatory cells – the so-called passenger leucocytes – that are believed to be important in initiating the allograft rejection process [10, 13], and their numbers increase markedly during the second trimester [17]. Culture of the HFP is commonly performed to deplete it of these cells prior to transplantation [13]. There has been no quantitative study of the effects of tissue culture on the accessory cell population of the HFP, but it is probable that the success of this manoeuvre depends on their initial density in the explants. The frequency of accessory cells in the HFP during the first trimester and the immunological profile of its other cells are not known as they have not been addressed adequately in previous studies [7, 9, 17, 24]. The present investigation was, therefore, carried out to rectify this hiatus in our knowledge of the immunology of the HFP.

Materials and methods

HFPs were harvested immediately after medically approved termination of pregnancy by the suction method. The specimens were embedded in OCT (BDH Chemicals, UK) without delay and snap-frozen in liquid nitrogen before being stored at −70°C. Ethical permission was granted for this study. Embryonic and foetal ages were determined by Streeter's developmental horizons [21] and foot length [19], respectively. Forty-six specimens aged 8–16 gw were analysed in total (Table 1). Although the distribution of class II antigens in HFPs older than 13 gw has been reported before [7, 9, 17, 24],

Table 1. Details of HFPs studied (n = 46)

Gestational age (weeks)	8	9	10	11	12	13	14	15	16
No. of specimens	2	8	9	9	4	5	5	1	3

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Table 2. Details of antibodies used

Monoclonal antibodies	Specificity	Dilution
1. Dakopatts M 736	HLA class I	1:320
2. Serotec MCA 354	HLA class I	1:25
3. Dakopatts M 704	HLA DR, DQ, DX	1:20
4. Serotec MCA 477	HLA DR, DP, DQ	1:50
5. MC119 (The Binding Site Ltd, Birmingham, UK)	Invariant chain	1:50
6. RFD 7 (Royal Free Hospital, UK)	Macrophages	1:50
7. Serotec FMC 32	Macrophages	1:50
8. Dakopatts M 755	B lymphocytes	1:25
9. Dakopatts M 756 (UCHT 1)	T lymphocytes	1:50
10. PAL E (Cambridge Bioscience, UK)	Endothelial cells	1:150
11. Dakopatts M 616	Von Willebrand factor	1:50
12. Dakopatts M 731	IL-2 receptor B chain	1:50
13. Anti – Leu 7 (Becton Dickinson)	NK cells Neuro-ectodermal tissue, pancreatic endocrine cells	1:20
14. FMC 16 (Sera-Lab)	Beta 2 microglobulin	1:50
Polyclonal antibodies		
Dakopatts A 072	Beta 2 microglobulin	1:100

second trimester specimens were included in the present series not only to ensure valid comparison in class II expression between the various age groups but also to detail the expression of class I antigens both before and after the 14th gw, as this is sparsely documented [7, 20]. Multispot microscopic slides were used and sequential cryostat sections 5 μm thick were cut; these were fixed in acetone at 4°C for 10 min and kept at room temperature for 24 h prior to being stained. Immunohistochemical staining was carried out using the two-stage indirect immunoperoxidase method [3] and, for class I antigen and beta 2 microglobulin (B2m), the APAAP technique was performed as well [8]. All of the primary antibodies used are listed in Table 2. Tonsillar tissue and older pancreases served as positive controls. Sequential sections, each stained for a single antigen, were examined. Typical antibody sequences included: PAL-E, class I and class II; Leu7, class I and class II; class I, B2m and class II; RFD7, FMC32 and class II. Cell counts were performed by examining five representative fields per specimen at 100× magnification.

Results

Class I expression

The pattern of class I antigen distribution was the same in HFPs aged 8–13 gw and in the older specimens. Both class I monoclonal antibodies used yielded similar results in all cases.

The blood supply of the HFP consisted of small arteries and veins and a well-defined capillary plexus of solid endothelial cell cords that was closely associated with the proliferating ductal system. The endothelial cells of all of these vascular structures were class I^+. Langerhans' islets were detected by 12 gw [15, 31] and they were identified by the monoclonal antibody Leu7, which cross-reacts with the pancreatic endocrine cells [6]. The islets expressed class I antigens. The ductal cells were $B2m^+$ when the polyclonal antibody, Dakopatts A 072, was used. However, the result was negative with the monoclonal antibody, FMC16 (Sera-Lab). Direct staining for class I antigens revealed the ductal cells to be negative (Fig. 1).

Table 3. Frequency of class II-positive cells in the HFP

Gestational age (weeks)	No. of class II-positive cells per 100× field
8	10
9	10
10	10
11	10
12	10
13	10
14	15
15	20
16	30

Class II expression

Identical results were obtained with both monoclonal antibodies used. The parenchymal cells of the HFP (ductal, exocrine and endocrine) and the endothelial cells were class II^- in all cases. Class II was detected in single cells with variable morphology that were present in the interstitial tissue (Fig. 2). Identification of these cells was carried out with monoclonal antibodies reactive against lymphocyte and macrophage markers. T lymphocytes were extremely rare and only a few B lymphocytes were observed. The majority of class II^+ cells were also positively stained with RFD7 and FMC32 monoclonal antibodies. Most of the $RFD7^+$ and $FMC\,32^+$ cells were, in fact, class II^-, while there were some class II^+ cells that were $RFD7^-$ and $FMC32^-$. The latter were presumably non-lymphoid dendritic cells [2], although cells with dendritic morphology were not restricted to this subset. In addition to these single cells, clusters of intensely class II^+ cells were detected in HFPs of all age groups. Macrophages and B lymphocytes were abundant, while only a smaller number of T cells were present. None of the cells in these aggregates expressed the receptor for interleukin-2.

In order to determine the influence of gestational age on the immunogenic potential of the HFP, the density of class II^+ cells in specimens aged 8–13 gw was comapred with that of HFPs aged 14–16 gw. The frequency of class II^+ cells remained constant during the first trimester. However, a threefold increase was observed from the end of the 13th gw to the 16th gw (Table 3).

The distribution of the invariant chain [22] matched that of class II antigens in all instances, and this excluded the possibility of adsorption causing spuriously positive results.

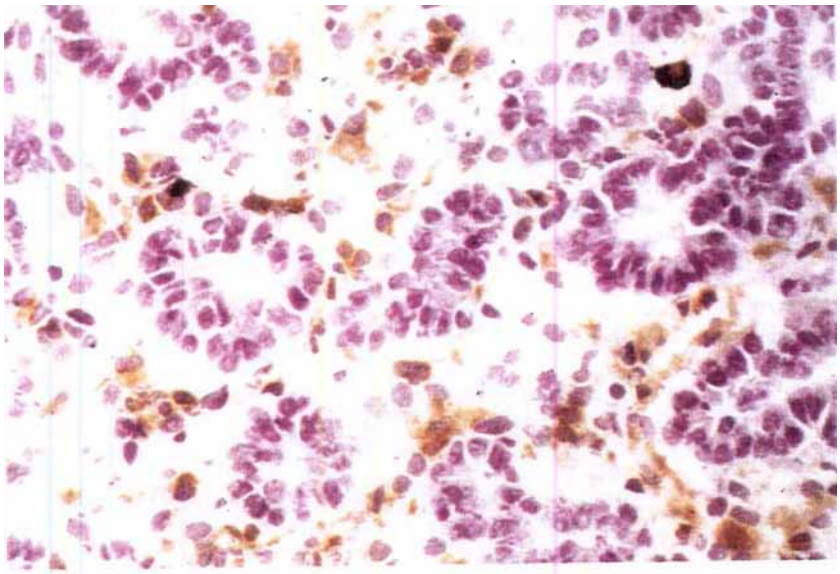

Fig. 1. Section of HFP showing class I^- ducts and class I^+ capillary cords using W6/32 (Dakopatts M 736; × 160)

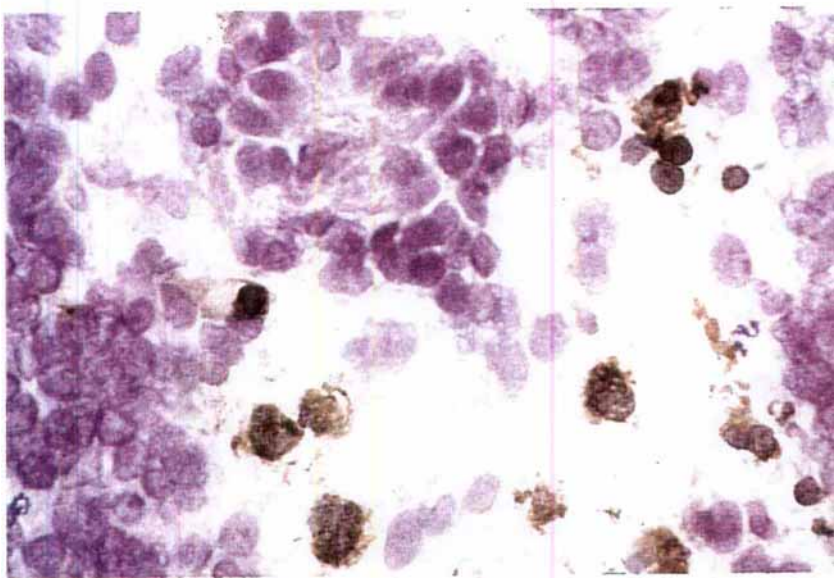

Fig. 2. Section illustrating the diverse morphology of the class II^+ cells in the HFP (Serotec MCA 477). Cells with lymphoid, macrophage and dendritic features are seen (× 400)

Discussion

The MHC antigens play a crucial role in the process of allograft rejection. Class II^+ cells, such as dendritic cells, macrophages and B lymphocytes, act as accessory or immunostimulatory cells to helper T lymphocytes, while class I^+ cells are the targets of cytotoxic T lymphocytes [1, 2, 25, 28]. The success of HFP transplantation depends on the continuing differentiation and development of endocrine cells from the primitive ductal tissue rather than on the few islets present in grafts at the time of the operation [4, 18]. The immunological properties of the ductal cells and their susceptibility to immune injury are thus crucial in determining the fate of HFP allografts. Surprisingly, the expression of class I antigens by the ductal cells has been neglected in previous immunological investigations of the HFP. Indeed, there are no such data on HFPs obtained during the first trimester, and the information on older HFPs is contradictory. Natali et al. have shown that the ductal epithelium of HFPs aged 20 and 26 gw were class I^- and $B2m^-$ using the monoclonal antibodies W6/32 and NAMB-1, respectively [20]. On the other hand, Cochrum et al. concluded that the ducts and islets of HFPs aged 16–22 gw were class I^+ on the grounds that they were $B2m^+$ [7]. These authors used a rabbit anti-human B2m polyclonal antibody (Sigma). With a similar antibody (Dakopatts 072), we obtained equally positive staining of the foetal pancreatic duct cells in specimens aged 8–16 gw. However, the results were negative in all cases when we used the B2m monoclonal antibody FMC16 (Sera-Lab). The reason for this discrepancy is unclear but is probably due to non-specific binding of the polyclonal antibody to the ductal cells. This view was supported by our results when class I antigen expression in those cells was assessed directly. Two monoclonal antibodies active against different epitopes of the class I molecule were utilised and both yielded negative results in all of the samples we analysed with the indirect immu-

noperoxidase and the APAAP methods. We, therefore, concluded that the ductal cells of the HFP were class I^-. These cells did not express the MHC class II antigens either.

The present report is the first comprehensive study of the phenotypes of class II^+ cells and their quantitative assessment in HFPs aged less than 13 gw. Previous authors have concentrated mainly on older HFPs [7, 9, 17, 24]. The existing data regarding the HFP during the first trimester are of limited value because they are either too sparse [9, 17] or based on the examination of a small number of unsuitable specimens retrieved long after prostaglandin-induced abortions [24].

Our investigations revealed that single class II^+ cells were found throughout the connective tissue of the HFP from the 8th gw onwards. The class II^+ cells included B lymphocytes and cells that were either positive or negative for the macrophage markers RFD7 and FMC32 as well. The expression of class II antigens by all of these cells was constitutive as they did not exhibit interleukin-2 receptors [12]. The ontogenic relationship between the so-called dendritic cell and the macrophage is unresolved [2]. There is no human dendritic cell marker, and its classification is normally based on the expression of class II antigens and morphological features. However, such criteria are not sufficiently discriminatory. In their study of accessory cells in the human foetus aged 4.5–22 gw (the pancreas was not included), Janossy et al. identified three distinct cell populations: $RFD7^+$, class II^- cells; $RFD7^+$, class II^+ cells and cells that were $RFD7^-$, class II^+ [11]. Significantly, all three groups contained cells with classical dendritic appearance.

The results of our analysis of class II^+ cells in the HFP are in agreement with these findings. Functionally, however, all three groups of cells, as well as the B lymphocytes, can contribute to tissue immunogenicity; the allograft rejection process is believed to be initiated by the dendritic cells, while the other accessory cells serve to amplify the reaction [2]. In addition to the cell types, the density of the accessory cell population within a graft is an important determinant of its immunogenic potential [23]. In the HFP aged 8–13 gw, the number of class II^+ interstitial cells was markedly less than in the second trimester. Thus, although the HFP is colonised by immunostimulatory cells from the 8th gw onwards, HFPs obtained during the first trimester would be better suited for immunomodulatory manoeuvres prior to transplantation.

Another characteristic feature of the HFP is the presence of groups of intensely class II^+ cells within its parenchyma. They were observed in HFPs of all ages and their size increased with gestational age. Danilovs et al. termed them T cell aggregates [9]. However, these authors only used antibodies against T lymphocyte antigens. With a wider range of antibodies, we found that the predominant cells in these clusters were macrophages and B lymphocytes, while T lymphocytes were, in fact, far less common. Furthermore, the T cells were unactivated (i.e. did not express interleukin-2 receptors) and, as such, unlikely to be class II^+ [27].

Our results indicate that the use of HFP grafts aged less than 14 gw might offer immunological as well as functional advantages compared with older HFPs in view of their lower numbers of accessory cells.

Acknowledgements. Financial support for this work was provided by the Special Trustees of Guy's Hospital. The authors also wish to thank the staff of the Obstetric Department, Dr. F. Compton of the Islet Research Laboratory and Miss J. Slater and Dr. B. Hartley of the Histopathology Department, Guy's Hospital, for their collaboration.

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