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# Regulation of major histocompatibility complex (MHC) products in fresh and cultured human fetal pancreata aged 9–16 gestational weeks by gamma-interferon

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**Abstract.** Immunological data on the human fetal pancreas (HFP) are mainly confined to its constitutive expression of the MHC antigens. However, cytokines, such as gamma-interferon (g-IFN), released by lymphocytes during immune reactions, can induce or upregulate the expression of MHC products in allografts and alter their immunological behaviour. We investigated the effects of g-IFN on fresh and cultured HFPs aged 9–16 gestational weeks (gw). Following g-IFN stimulation of fresh HFPs, there was class I hyperexpression by the ductal cells, and some of the ductal, endothelial and islet cells also became class II$^+$. Conventional tissue culture (5% $CO_2$ in air at 37 °C) reduced the number of interstitial class II$^+$ cells within the HFP after 1 week but was associated with de novo class I expression by some of the ductal cells. Remarkably, the changes in major histocompatibility complex (MHC) antigen expression by the ductal cells occurred earlier and were markedly enhanced when the HFPs were cultured beforehand. The number of interstitial class II$^+$ cells in fresh and cultured HFPs was not influenced by g-IFN. The significance of these observations with regard to clinical HFP transplantation is discussed.

**Key words:** MHC regulation, human fetal pancreas – Fetal pancreas, human, MHC regulation – Gamma-interferon, MHC regulation, human fetal pancreas – Pancreas, human fetal, MHC regulation

Major histocompatibility complex (MHC) products play a central role in the immune process. CD4$^+$ helper T lymphocytes are activated by class II$^+$ accessory cells while class I antigens are the main targets of CD8$^+$ cytotoxic T lymphocytes [4]. Our studies and those of other groups have shown that class II$^+$ immunostimulatory cells are present in the human fetal pancreas (HFP) starting from the 8th gestational week (gw) and that their numbers increase markedly from the end of the first trimester onwards [3, 5, 12, 15, 18]. It is thus not surprising that the HFP can trigger the rejection process. On the other hand, it is less clear how immune damage to the graft is mediated. The endothelial cells and islets are class I$^+$, but class I and class II antigens cannot be detected on the primitive ductal cells of the HFP [15]. Successful transplantation of the HFP depends on the survival and growth of these ductal cells as they are the precursors of the pancreatic endocrine cells [6]. Their lack of class I expression would be expected to protect the ductal cells from destruction by class I-restricted cytotoxic T lymphocytes and to favour their successful engraftment. The evidence suggests, however, that following clinical transplantation, the ductal cells of the HFP are, in fact, rejected [29]. Intra-graft events post-transplantation are likely to be involved in determining the fate of the HFP. The local release of cytokines by activated T lymphocytes can significantly alter the immunogenic profile of an allograft and have a strong influence on its survival [10, 26]. The effects of one of these mediators, gamma-interferon (g-IFN), on class I and class II antigen expression by lymphoid and non-lymphoid cells are well documented [24, 25]. In the first part of the present study, we analysed the modulation of MHC antigen expression by the cells of the fresh HFP by g-IFN.

Preliminary tissue culture of the HFP under conventional conditions for varying periods of time has frequently been used in clinical transplantation studies as a means of reducing its immunogenicity by depleting it of immunostimulatory cells [1, 9, 13, 32]. Nevertheless, the effects of the procedure on the immunological profile of the HFP have not been previously analysed. We therefore investigated this aspect of HFP immunobiology and, in addition, whether conventional tissue culture influenced the subsequent response of the HFP to g-IFN.

## Materials and methods

HFPs were retrieved immediately after mechanical abortions. Ethical permission was granted for this study. Gestational age was estimated by foot length [19].

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**Table 1.** Details of HFPs analysed

| Age (gestational weeks) | Group 1 ($n = 16$) | Group 2 ($n = 46$) | Group 3 ($n = 9$) |
|---|---|---|---|
| 8–13 | 13 | 29 | 6 |
| 14 | 2 | 8 | 3 |
| 15 | – | 2 | – |
| 16 | 1 | 7 | – |

### *Stimulation of fresh HFPs by g-IFN (group 1)*

Sixteen fresh HFPs aged 9–16 gw were studied (Table 1). Each specimen was divided into 1-mm$^3$ pieces and the fragments were placed into two non-treated Petri dishes containing 2.5 ml of RPMI 1640, supplemented with L-glutamine (300 mg/l), antibiotics (100 u/ml benzylpenicillin and 100 µg/ml streptomycin) and 10% heat-inactivated male AB human serum. The following protocol was used to determine the effective dose of g-IFN and the period of stimulation necessary to obtain a significant response in terms of induction and upregulation of MHC antigen expression. The contents of one Petri dish were stimulated with 50 u/ml of human g-IFN for 1–4 days while the other half of the sample was stimulated for the same number of days with 200 u/ml of g-IFN. As in all the other experiments carried out in this study, purified human g-IFN produced from buffy coats by induction with A23187 and mezerein was used (Sigma Chemicals, Dorset, UK). The culture medium was changed daily and freshly prepared g-IFN solution was then added as well. The explants were maintained in an atmosphere of 5% $CO_2$ in humidified air at 37 °C throughout the course of these experiments.

### *Effects of conventional tissue culture on the HFP (group 2)*

For the second part of the study, 46 HFPs aged 9–16 gw were maintained in conventional tissue culture conditions as described above for 1–2 weeks (Table 1). The culture medium was changed daily.

### *Stimulation of 1-week-cultured HFPs (group 3)*

Nine HFPs aged 10–14 gw were used (Table 1). These were first maintained for 1 week in the conventional culture conditions before they were stimulated by g-IFN. Based on the dose-response data derived from fresh HFPs, the 1-week-cultured explants were subsequently exposed to 200 u/ml of g-IFN for 1–4 days. The culture medium was changed every 24 h and fresh g-IFN was added at the same time.

HFP fragments in all three groups were harvested and dried on a piece of blotting paper before they were embedded in OCT (BDH Chemicals, Poole, UK) and snap-frozen in liquid nitrogen. Viability of all the specimens included in the study was first ascertained by examining sections stained with haematoxylin and eosin. Histological studies were performed on 5-µm-thick sequential cryostat sections singly stained using the two-stage indirect immunoperoxidase method [2]. The primary monoclonal antibodies utilised are listed in Table 2. Counts of class II$^+$ cells were performed by analysing five representative fields per specimen at 100× magnification and the mean values were calculated.

## Results

In fresh HFPs, the ductal cells were class I$^-$ and class II$^-$, as we have reported previously [15]. g-IFN-induced class I expression by the ductal cells of fresh HFPs and the response, in terms of the number of class I$^+$ cells and the intensity of staining, was dependent on the dose of cytokine used and on the duration of the stimulation period. About 50% of the ducts expressed low levels of class I antigens after stimulation with 50 u/ml of g-IFN for 1 day (Fig. 1). After incubation with 50 u/ml of g-IFN for 4 days, de novo class I and beta 2 microglobulin expression was observed in up to 75% of the ducts and the intensity of the staining was somewhat stronger. Following exposure of the tissue to 200 u/ml of g-IFN for 1 day, the changes closely resembled those induced by 50 u/ml of g-IFN for 4 days. In both cases, the ductal cells remained class II$^-$. The greatest effects were seen after stimulation of the fresh HFPs with 200 u/ml of g-IFN for 4 days. All the ducts were class I$^+$ and, in most of the cells, the staining was strikingly more intense than previously observed (Fig. 2). Some of the ducts also became class II$^+$ and the degree of expression was usually weak (Fig. 3). The capillary endothelial cells remained class II$^-$, although class II induction was seen in the endothelial cells of some of the larger vessels of the 16 gw specimens. Occasional class II$^+$ islets were also seen.

After 1 week in conventional tissue culture, there was a reduction in the number of interstitial class II$^+$ cells in the HFPs (Table 3). This was more marked in first trimester HFPs. Extending the culture period to 2 weeks did not deplete these cells further. In the cultured HFPs, de novo class I antigens could be detected in some of the previously class I$^-$ ductal cells (Fig. 4). There was no evidence of concomitant induction of class II antigens in these cells.

Stimulation of HFPs cultured for 1 week first revealed that the sensitivity of the tissue to g-IFN was markedly increased. Using 200 u/ml of g-IFN, intense expression of class I antigens – more marked than in fresh HFPs – was detected after only 24 h in all the ductal cells (Fig. 5). A few of these cells expressed class II products as well. In cultured HFPs stimulated with 200 u/ml of g-IFN for 4 days, up to 50% of the ductal cells became class II$^+$, as compared to less than 5% in fresh HFPs treated similarly. The staining for class II antigens was generally stronger in the cultured HFPs (Fig. 6). The larger vessels were lost during culture, and the surviving capillary endothelial cells remained class II$^-$. Some class II$^+$ islets were detected.

In both fresh and cultured HFPs, no increase in the number of class II$^+$ accessory cells was detected after g-IFN stimulation (Table 3), although RFD7$^+$ class II$^-$ macrophages were present in the specimens.

**Table 2.** Details of monoclonal antibodies used

| Monoclonal antibody | Specificity | Titre |
|---|---|---|
| 1. Dakopatts M 736 | HLA class I | 1:320 |
| 2. Serotec MCA 354 | HLA class I | 1:25 |
| 3. FMC 16 (Sera-Lab) | Beta 2 microglobulin | 1:50 |
| 4. Dakopatts M 704 | HLA DR, DP, DX | 1:20 |
| 5. Serotec MCA 477 | HLA DR, DP, DQ | 1:50 |
| 6. MC 119 (The Binding Site, Birmingham, UK) | Invariant chain | 1:50 |
| 7. PAL E (Cambridge Bioscience, UK) | Endothelial cells | 1:150 |
| 8. Anti-Leu7 (Becton-Dickinson) | NK cells<br>Neuro-ectodermal tissue<br>Islet cells | 1:20 |
| 9. RFD7 (Royal Free Hospital, UK) | Macrophages | 1:50 |

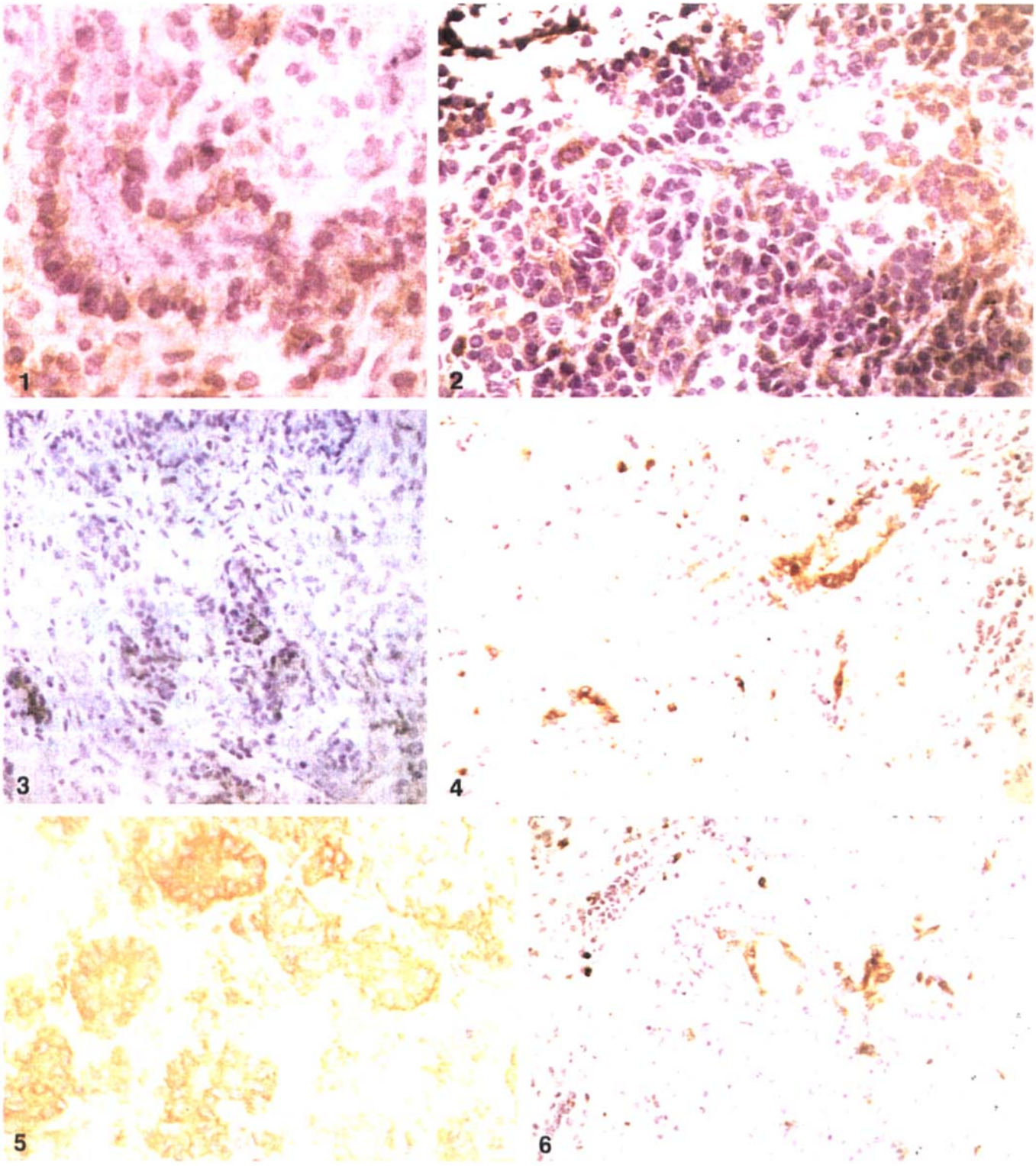


**Fig. 1.** Class I$^+$ duct in fresh HFP after stimulation with 50 u/ml of g-IFN for 24 h (Dakopatts 736, × 400)

**Fig. 2.** High levels of class I expression by most of the ductal cells in fresh HFP after stimulation with 200 u/ml of g-IFN for 4 days (Dakopatts 736, × 160)

**Fig. 3.** Class II expression by the ductal cells after stimulation of fresh HFP with 200 u/ml of g-IFN for 4 days (Serotec MCA 354, × 100)

**Fig. 4.** De novo class I expression by ductal cells in HFP after 1 week of conventional tissue culture (Dakopatts M 736, × 100)

**Fig. 5.** Hyperexpression of class I antigens in cultured HFP after stimulation with 200 u/ml of g-IFN for only 24 h (Dakopatts M 736, × 160)

**Fig. 6.** Cryptic class II antigen expression in cultured HFP after exposure to 200 u/ml of g-IFN for 4 days (Serotec MCA 354, × 100)

**Table 3.** Mean density of class II$^+$ cells

| Age (gestational weeks) | Fresh HFPs | Group 1[a] | Group 2 | | Group 3[b] |
|---|---|---|---|---|---|
| | | | 1 week | 2 weeks | |
| 8–13 | 10 | 10.5 | 2.5 | 2.5 | 2.5 |
| 14 | 15 | 15 | 6.5 | 5.5 | 7.0 |
| 15 | 20 | – | 10 | 10 | – |
| 16 | 30 | 29 | 15 | 13.5 | – |

[a] Fresh HFPs stimulated with 200 u/ml of g-IFN for 4 days
[b] 1-week-cultured HFPs stimulated with 200 u/ml of g-IFN for 4 days

The changes in MHC antigen levels seen after g-IFN stimulation, except for the selective induction of class II expression observed in the endothelial cells of the large vessels in 16 gw fresh HFPs, were not influenced by the gestational age of the sample. The distribution of beta 2 microglobulin and of the invariant chain matched that of their respective MHC molecule.

## Discussion

The potential value of HFP allotransplantation in the treatment of insulin-dependent diabetes mellitus is often discussed, but its role remains to be properly defined [1]. A major obstacle to the success of the procedure is rejection. Although a large number of HFP grafts have been performed in humans [1, 13], disappointingly little is known about their immunological behaviour.

In the present study, the post-transplantation milieu was mimicked by stimulating the HFP with g-IFN. The results provide greater insight into the immunogenic properties of the fresh HFP. The hyperexpression of class I antigens after exposure to g-IFN by the previously class I$^-$ ductal cells emphasises the limitations of immunological concepts based on the constitutive expression of MHC antigens. Following cytokine release during the rejection process, they are likely to become susceptible to destruction by class I-restricted cytotoxic T lymphocytes. The fact that colonisation of the HFP by class II$^+$ accessory cells occurs from very early on during its development [15] and our present findings regarding the inducible nature of class I antigen expression by its parenchymal cells do not support claims that the tissue is poorly immunogenic or is an immunologically protected target [7].

In addition to its effects on class I expression, g-IFN affected the regulation of class II antigens in fresh HFPs. Some ductal cells became class II$^+$, as did the endothelial cells of some of the large vessels in the sample aged 16 gw. Gestational age has an influence on the expression of class II antigens by the endothelial cells of fresh, unstimulated HFPs. Our own data in HFPs aged 8–16 gw have shown that class II antigens could not be detected in the endothelial cells in these age groups [15] and, in their analysis of HFPs aged 12–24 gw, Motojima et al. [18] found that class II$^+$ endothelial cells were only present in specimens older than 18 gw. Such cells were initially found in small vessels and the endothelia of medium-sized vessels only became class II$^+$ by the 24th gw. The capillary endothelial cells remained class II$^-$. Our findings that stimulation of the fresh HFP with g-IFN caused the endothelia of some of the large vessels in HFPs aged 16 gw to express class II antigens are also in agreement with the observations of these authors. Class II expression by the endothelial cells can endow them with antigen-presenting function [21], and this might also account in part for the immunogenicity of the HFP following its allotransplantation. The role of the class II$^+$ endocrine and ductal cells in the initiation of the rejection process is not known, but they could be the targets of class II-restricted cytotoxic T lymphocytes [28].

There is only one previous report on the effects of tissue culture on the interstitial class II$^+$ cell populations of the HFP [27]. The specimens were obtained 4–6 h after prostaglandin-induced abortions and tissue culture was carried out in high levels of oxygen. Unlike HFPs retrieved after mechanical abortions, samples harvested after prostaglandin-induced abortions are often nonviable [20]. Furthermore, high levels of oxygen are toxic to the HFP [14, 16], while incubation in 5% $CO_2$ in humidified air at 37 °C is well tolerated by the tissue, as evidenced by its continuing development and differentiation [8, 16, 22, 33]. However, the immunomodulatory effects of such culture conditions are unknown. Our findings that the number of class II$^+$ interstitial cells was lower after conventional tissue culture supports the view that the procedure might confer some immunological benefit if it is performed prior to allotransplantation. The mechanisms involved in the selective loss of these cells are unknown. It is not dependent on the use of bovine serum [23], as we used human serum exclusively in all our experiments. The reason for the greater depletion of the interstitial class II$^+$ cells in first trimester HFPs is also not clear, but this observation is pertinent to HFP allotransplantation, bearing in mind their superior growth potential in vitro and in vivo [31, 33].

De novo expression of MHC antigens during tissue culture has been reported in other cell types [11, 17]. This phenomenon might be related either to the presence of a stimulating factor or the absence of an inhibitor in the added serum or, alternatively, to the production of mediators by macrophages phagocytosing cellular debris. Whatever its cause, the consequences of this alteration in class I expression by the HFP are likely to be deleterious to the survival of such grafts post-transplantation.

The potential risks of pretreatment by conventional tissue culture were further emphasised by the results of g-IFN stimulation of such tissue. The cultured HFPs were strikingly more sensitive to the actions of the cytokine. Compared to fresh HFPs, stronger expression of class I antigens by the ductal cells occurred much earlier and was seen in all these cells. In addition, a greater number of the ductal cells became class II$^+$ by day 4 in the cultured HFPs stimulated with 200 u/ml of g-IFN. It would appear that the HFPs had been conditioned during tissue culture.

The number of class II$^+$ interstitial cells (accessory cells) in many organs is increased following g-IFN stimulation [24]. We noted no such changes in the fresh and cultured HFPs that we studied. Similar results have been reported by others in fresh HFPs and in HFPs cultured for 4 days only before g-IFN stimulation [18]. It might be that the macrophage population of the HFP consists of estab-

lished cells; unlike young monocytes, "old" tissue macrophages appear to be resistant to the actions of g-IFN [30].

In conclusion, our results provide further new evidence that the HFP is not immunologically privileged. More importantly, they highlight the fact that although significant accessory cell depletion was observed in HFPs maintained in conventional tissue culture conditions, it was incomplete even after 2 weeks. Paradoxically, initiation of the rejection process after allotransplantation of cultured HFPs might, in fact, lead to the accelerated immune destruction of these grafts in view of their enhanced responsiveness to g-IFN. These potentially harmful effects of immunomodulation by conventional tissue culture have not been reported previously.

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## References

1. Andersson A, Sandler S, Groth CG, Hellerstrom C (1988) Transplantation of fetal islet tissue. In: Groth CG (ed) Pancreatic transplantation. Saunders, Philadelphia London Toronto, pp 391–406
2. Bourne JA (1983) Handbook of immunoperoxidase staining methods. Dako Corporation, Santa Barbara
3. Cochrum HC, Jemtrud S, Lim RC, Hunt TK, Parry G (1989) MHC antigens persist in the human fetal pancreatic islet cells even after culture and transplantation into nude mice. Transplant Proc 21: 2653–2656
4. Cramer DV, Gill TJ III (1986) Biology of disease: Genetic aspects of cellular interactions in the immune response. Lab Invest 55: 126–137
5. Danilovs JA, Hofman FM, Taylor CR, Brown J (1982) Expression of HLA-DR antigens in human fetal pancreas tissue. Diabetes 31 [Suppl 4]: 23–28
6. Dudek RW, Freinkel N, Lewis NJ, Hellerstrom C, Johnson RC (1980) Morphologic study of cultured pancreatic fetal islets during maturation of the insulin stimulus-secretion mechanism. Diabetes 29: 15–21
7. Eloy R, Doillon C, Dubois P (1980) Fetal pancreatic transplantation- review of experimental data. Transplant Proc 12 [Suppl 2]: 179–185
8. Farkas G, Joo F (1984) Simple and reliable conditions for routine, long-term culturing of fetal human pancreatic tissue fragments. Diabetes 33: 1165–1168
9. Groth CG, Andersson A, Bjorken C, Gunnarsson R, Hellerstrom C, Peterson B, Swenne I, Ostman J (1980) Transplantation of fetal pancreatic microfragments via the portal vein to a diabetic patient. Diabetes 29 [Suppl 1]: 80–83
10. Hall BM (1987) Cellular infiltrates in allografts. Transplant Proc 19: 50–56
11. Halloran P, Wadgymar A, Autenfield P (1986) The regulation of expression of major histocompatibility complex products. Transplantation 41: 413–420
12. Hofman FM, Danilovs JA, Taylor CR (1984) HLA-DR (Ia)-positive dendritic-like cells in human fetal nonlymphoid tissues. Transplantation 37: 590–594
13. Hu Y-F, Gu Z-F, Zhang H-D, Ye R-S (1989) Fetal islet cell transplantation in China. Transplant Proc 21: 2605–2607
14. Hullett DA, Landry AS, Leonard DK, Sollinger HW (1989) Improved human fetal pancreatic tissue survival following hyperbaric oxygen culture. Transplant Proc 21: 2659–2660
15. Koo Seen Lin LC, Welsh KI, Koffman CG, McColl I (1991) The immunology of the human foetal pancreas aged 8–13 gestational weeks. Transplant Int 4: 195–199
16. Mandel TE, Hoffmann L, Collier S, Carter WM, Koulmanda M (1982) Organ culture of fetal mouse and fetal human pancreatic islets for allografting. Diabetes 31 [Suppl 4]: 39–47
17. Mauerhoff T, Pujol-Borel R, Mirakian R, Bottazo GF (1988) Differential expression and regulation of major histocompatibility complex (MHC) products in neural and glial cells of the human fetal brain. J Neuroimmunol 18: 271–289
18. Motojima K, Matsuo S, 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
19. Munsick RA (1984) Human fetal extremity lengths in the interval from 9 to 21 menstrual weeks of pregnancy. Am J Obstet Gynecol 149: 883–887
20. Otonkoski T, Wong I, Goldman H, Knip M, Andersson A, Panula P, Simell O (1986) Yield and functional integrity of islet-like cell clusters in culture of human fetal pancreata from hysterotomy, mechanical and prostaglandin-induced abortions. Diabetologia 29: 579A
21. Pober JS, Collins T, Gimbrone MA, Libby P, Reiss CS (1986) Inducible expression of Class II major histocompatibility complex antigens and the immunogenicity of vascular endothelium. Transplantation 41: 141–145
22. Sandler S, Andersson A, Schnell A, Mellgren A, Tollemar J, Borg H, Petersson B, Groth CG, Hellerstrom C (1985) Tissue culture of human fetal pancreas. Development and function of B-cells in vitro and transplantation of explants to nude mice. Diabetes 34: 1113–1119
23. Serie JR, Hickey GE, Schmitt RV, Hegre OD (1983) Prolongation of culture-isolated neonatal islet xenografts without immunosuppression. Transplantation 36: 6–11
24. Skoskiewicz M, Colvin RB, Schneeberger EE, Russell PS (1985) Widespread and selective induction of major histocompatibility complex-determined antigens in vivo by Gamma-interferon. J Exp Med 162: 1645–1664
25. Steeg P, Moore RN, Johnson HM, Oppenheim JJ (1982) Regulation of murine macrophage Ia antigen expression by a lymphokine with immune interferon activity. J Exp Med 156: 1780–1793
26. Steiniger B, Klempnauer J, Wonigeit K (1985) Altered distribution of Class I and Class II MHC antigens during acute pancreas allograft rejection in the rat. Transplantation 40: 234–239
27. Thomson NM, Hancock WM, Lafferty KJ, Kraft N, Atkins RC (1983) Organ culture reduces Ia-positive cells present within the human fetal pancreas. Transplant Proc 15: 1373–1376
28. Thorsby E (1987) Structure and function of HLA molecules. Transplant Proc 19: 29–35
29. Tuch BE, Sheil AGR, Ng ABP, Trent BJ, Turtle JR (1988) Recovery of human fetal pancreas after one year of implantation in the diabetic patient. Transplantation 46: 865–870
30. Unanue ER (1984) Antigen-presenting function of the macrophage. Annu Rev Immunol 2: 395–428
31. Usadel KH, Schwedes G, Bastert G, Steinau U, Klempa I, Fassbinder W, Schoffling K (1980) Transplantation of human fetal pancreas. Experience in thymus aplastic mice and rats and in a diabetic patient. Diabetes 29 [Suppl 1]: 74–79
32. Valente U, Ferro M, Barocci C, Campisi C, Parodi F, Cataldi L, Arcuri V, Tosatti E (1980) Report of clinical cases of human fetal pancreas transplantation. Transplant Proc 12 [Suppl 2]: 213–217
33. Von Dorsche HH, Reiher H, Hahn H-J, Falt K, Falkmer S (1987) Tissue cultivation as a method for preservation of human fetal islet parenchyma- a correlated biochemical, immunohistochemical and morphometric investigation. Diab Res 5: 157–161