Diabetologia (1992) 35: 63–69

Diabetologia

Mumps virus infects Beta cells in human fetal islet cell cultures upregulating the expression of HLA class I molecules

P. Parkkonen[1], H. Hyöty[1], L. Koskinen[2] and P. Leinikki[3]

[1] Institute of Biomedical Sciences, University of Tampere, [2] Tampere University Hospital, Tampere, and
[3] National Public Health Institute, Helsinki, Finland

Summary. The ability of mumps virus to infect pancreatic Beta cells and cause alterations in their HLA expression was evaluated in cultured human fetal islet cell clusters. Mumps virus could be isolated during the whole culture period (6–8 days) and 60% of cells, including Beta cells, contained viral nucleocapsid protein at the end of the culturing. A minor decrease in insulin secretion was observed in some of the infected cultures. The infection was invariably associated with an increase in the expression of HLA class I molecules. This enhancement was mediated by soluble factors secreted by infected cells. The infection could not induce the expression of HLA-DR molecules. However, external interferon-gamma was able to cause a clear rise in DR-expression which was observed only on non-Beta-cells. Rubella and coxsackie B4 viruses were also able to enhance the expression of class I molecules while herpes simplex virus type 2 was not. The results suggest that certain viruses are able to infect Beta cells and cause alterations in their immunological appearance. Increased HLA class I expression in infected islets may exaggerate the autoimmune process in pre-diabetic individuals by increasing the activity of autoreactive cytotoxic T cells.

Key words: Islet cells, mumps virus, HLA molecules.

Some viruses have been suspected of being involved in the T-cell-mediated destruction of pancreatic Beta cells during the prediabetic stage of Type 1 (insulin-dependent) diabetes [1, 2]. Different mechanisms of action have been implicated such as direct lysis of cells by target-cell specific cytolytic virus strains [3] or induction of immunological destruction as a result of molecular mimicry [4] or altered immunological appearance of Beta cells [5]. One possibility is that virus infection induces enhanced expression of HLA molecules on Beta cells thus promoting the presentation of Beta-cell autoantigens to the immune system [6]. Previously, mumps and coxsackie B viruses, both being suspected of playing a role in the pathogenesis of Type 1 diabetes, have been shown to infect Beta cells in vitro [7, 8] but very little is known about their ability to cause aberrant HLA expression on these cells. In the present investigation human fetal islet cell cultures were used to study the ability of mumps, coxsackie B4 virus (CB4), rubella and herpes simplex virus type 2 (HSV-2) viruses to infect islet cells and their effects on the insulin secretion and the HLA expression of Beta cells.

Materials and methods

Cell cultures

For islet cell cultures human fetal pancreases were taken after legally approved abortions performed for social indications by prostaglandin induction. The gestational age of the fetuses was 12 to 17 weeks. The use of pancreatic tissue was approved by the local ethical committee according to the instructions of the Finnish Medical Council. Islet cell cultures were prepared by collagenase digestion from mechanically disrupted pancreas tissues as previously described [9, 10]. Endocrine cells were allowed to form free-floating islet cell clusters on a polycarbonate net in plastic tissue culture inserts (Costar Transwell Cell Culture Inserts, Costar Europe Ltd., Badhoevedorp, The Netherlands) during 24 h incubation at +37 °C in RPMI 1640 (Gibco, New York, NY, USA) supplemented with 10% inactivated fetal calf serum (FCS, Gibco), somatotropin 1 μg/ml, glutamine and antibiotics. After 2 days of culture, FCS was replaced by inactivated human AB-serum in the medium.

Human embryonic lung fibroblast cultures were prepared from fetal lung cells by treating tissue with 0.2% trypsin solution. Fibroblast cultures were grown in hepes-DMEM (Dulbecco's Modification of Eagle's Medium, Gibco) with 10% FCS, glutamine and antibiotics. All cell cultures were grown in humidified 95% air/5% CO_2 at +37 °C (standard cell culture conditions).

Virus infections

Human islet cell cultures were infected on day 3 with mumps virus (Enders-strain). Virus stock was grown in Vero cells and virus inoculum contained 0.3 ml of this stock supplemented by 2.7 ml of culture medium containing 2% FCS. The islet cells were infected as free-floating clusters on the cell inserts using 1 h absorption at +37 °C. After incubation the medium was discarded and replaced with RPMI 1640 supplemented with 2% human serum, glutamine and antibiotics. Infected islets were cultured for 6 days with daily changes of culture medium. Mock-infected cultures were handled

analogously. In some experiments the cultures were infected with CB4 (WHO-strain), rubella (Therien-strain) or HSV-2 (strain G) viruses using the same protocol. For some experiments mumps virus inoculum was inactivated by heating for 1 h in + 56 °C water bath.

Virus replication in islet cells was evaluated by titrating the daily collected culture mediums in Vero-cells (ATCC CRL 1586) for the presence of infective virus. Vero-cells were grown in 24 tissue culture clusters (Costar) as confluent monolayer in Eagle's Modified Medium (Gibco) supplemented with 2% newborn bovine serum (Flow Laboratories, Irvine, Scotland, UK), glutamine, non-essential amino acids, glucose and antibiotics. Cultures were infected with islet cell supernatants as above, grown for 5 or 6 days and viewed daily for cytopathic effect (CPE).

Flow-cytometric analysis of HLA expression

The intensity of HLA molecules was studied by immunofluorescence methods from unfixed cells. Islet cell clusters were first washed 3 times with phosphate buffered saline (PBS) and disrupted by 0.2% trypsin solution using 15 min incubation in + 37 °C water bath. After incubation the one cell suspension was pelleted by centrifugating for 10 min in 1200 *g* using Heraeus Labofuge GL (Heraeus Separationstechnik, Osterode, FRG) and washed twice with PBS. HLA class I molecules were stained by an anti-HLA-A,B,C antibody M736 (Dako Immunoglobulins, Copenhagen, Denmark) in appropriate dilutions in PBS supplemented with 2% FCS and 0.02% NaN_3. Fluorescein isothiocyanate-(FITC) labelled anti-mouse IgG (Dako) was used as the secondary antibody. HLA class II molecules were stained using a monoclonal phycoerythrin (PE) conjugated anti-HLA-DR antibody (Becton Dickinson No. 7367, Mountain View, Calif., USA). The expression of class I antigens was quantitated according to the intensity of green fluorescence (the mean fluorescence channel number) and class II molecules according to the red fluorescence in flow-cytometry (FACScan, Becton Dickinson). Propidium iodide (Sigma, St. Louis, Mo., USA) was added to the cell suspension (25 µg/10^6 cells) to exclude dead cells on the basis of red fluorescence. In some experiments the number of T lymphocytes in islet cell suspension was evaluated using FITC-conjugated antibodies to CD3 (Leu 4, Becton Dickinson) and the number of monocytes using antibodies to CD14 (Mo2, Coulter Corporation, Hialeah, Fl., USA) and FITC-conjugated anti-mouse immunoglobulins (Dako). Fibroblasts were detached with 0.2% trypsin solution and stained according to the same formula.

Immunohistochemistry

Islet cell clusters were disrupted into one cell suspension as above and fixed with 2% paraformaldehyde-PBS solution at + 4 °C for 3 min on microscope slides. Cells containing both insulin and mumps virus nucleocapsid protein were visualized by double immunofluorescence staining using simultaneous incubation of guinea-pig anti-insulin antibodies (Dako) in 1:100 dilution and a mouse monoclonal antibody specific for mumps virus nucleocapsid protein in 1:200 dilution in PBS containing 1% bovine serum albumin (BSA) and 0.3% Triton X-100. This monoclonal nucleocapsid specific antibody (Cl. 5.495), kindly provided by Dr. C. Örvell (Statens Bacteriologiska Laboratorium, Stockholm, Sweden), has been raised against egg-grown mumps virus and shown to be specific for the nucleocapsid protein [11]. Rhodamine conjugated goat anti-guinea-pig antibodies (Cappel, Organon Teknika Corp., Treyburn, NC, USA) as well as biotin-labelled anti-mouse antibodies (Amersham, Sydney, Australia) and FITC-conjugated avidin (Vector Laboratories, Burlingame, Calif., USA) were used as the second layer, respectively. The amount of positive cells was calculated by counting at least 2 times 400 cells using Leitz Aristoplan microscope with appropriate filters to fluorescein and rhodamine. HSV-2 virus was detected from infected islet cells by FITC-conjugated monoclonal antibodies against HSV-2 virus (Syva Company, Palo Alto, Calif., USA). HLA class II molecules were stained on microscope slides as for flow-cytometry using anti-HLA-DR alpha chain antibodies specific for epitopes resistant to the paraformaldehyde fixation (M746, Dako).

Other methods

The effects of interferon (IFN)-gamma and tumour necrosis factor (TNF) on HLA expression were studied by adding recombinant human IFN-gamma (Genentech, San Francisco, Calif., USA) alone or in combination with human TNF-alpha (Genzyme, Boston, Mass., USA), both in concentrations of 1000 U/ml, to the culture mediums on day 3. The possible interactions of lymphokines and mumps virus on HLA expression was analysed by adding 1000 U/ml of IFN-gamma to the culture supernatants after virus absorption.

To evaluate the role of soluble factors in HLA induction part of supernatants from mumps virus or mock-infected islet cell cultures were collected at day 4–6 and depleted from infective virus using 2-h, ultraviolet-irradiation at room temperature. Fibroblast culture mediums were replaced by inactivated islet cell supernatants and after 1 h incubation at + 37 °C standard 2% FCS-DMEM medium was added to these supernatants (1:3). The density of HLA antigens was evaluated after 5 or 6 days culturing in flow-cytometry. Inactivation of the virus was confirmed by the lack of CPE in fibroblast cultures after 7 or 8 days culture period.

The concentration of immunoreactive insulin was analysed by a commercial radioimmunoassay kit (Human insulin RIA kit, Novo Biolabs, Baegsvaerd, Denmark) from the culture medium serially collected from virus and mock-infected islet cell cultures and stored at − 70 °C until tested.

Statistical analysis

Student's *t*-test was used when statistical significances between infected and non-infected cultures were evaluated.

Results

Mumps virus infection caused no visible CPE in pancreatic islet cell clusters (Fig. 1 A) and in flow-cytometry the cell-size and granularity parameters were quite similar in infected and mock-infected cultures. The average of 5% of cells contained immunoreactive insulin and similar proportions of cells were also positive for glucagon and somatostatin. Anti-CD3 and -CD14 antibodies showed no binding to islet cells in flow-cytometry suggesting that T lymphocytes or monocytes were not contaminating these cultures.

Mumps virus seemed to be able to replicate in islet cells as infective virus could be isolated from culture supernatants throughout the 6–8 days' infection period (six experiments). At the end of the culturing mumps virus nucleocapsid protein was found by indirect immunofluorescence in about 60% of islet cells and in double-fluorescence staining in a comparable proportion of insulin cells (Fig. 1 B, C). Infection had no effect on the total number of insulin-positive cells during the culture period.

Insulin concentration was analysed from the culture supernatants collected serially from a total of six mumps virus infected islet cell cultures and from six uninfected control cultures (Fig. 2). In two of these experiments insulin concentration rapidly decreased after infection and

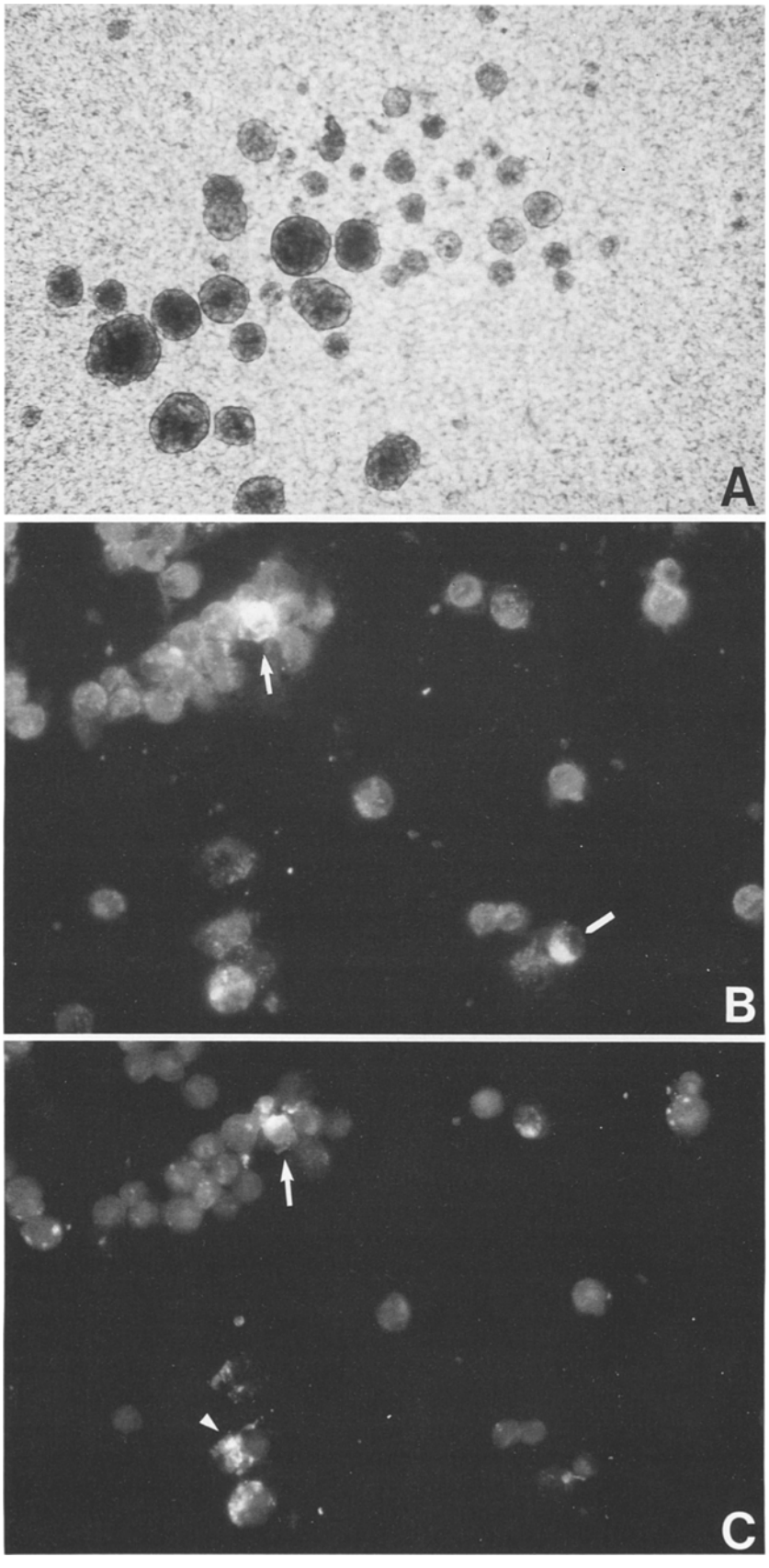


Fig. 1. **A** shows free-floating islet cell clusters 3 days after isolation from the fetal pancreas. **B** and **C** present double immunofluorescence stainings of single cell suspension from islet cell clusters 6 days after infection by mumps virus. The cells containing both immunoreactive insulin (**B,** rhodamine fluorescence) and mumps virus nucleocapsid proteins (**C,** fluorescein isothiocyanate-fluorescence), are indicated by large arrows. Cells positive for only insulin or only nucleocapsid protein are indicated by small arrows

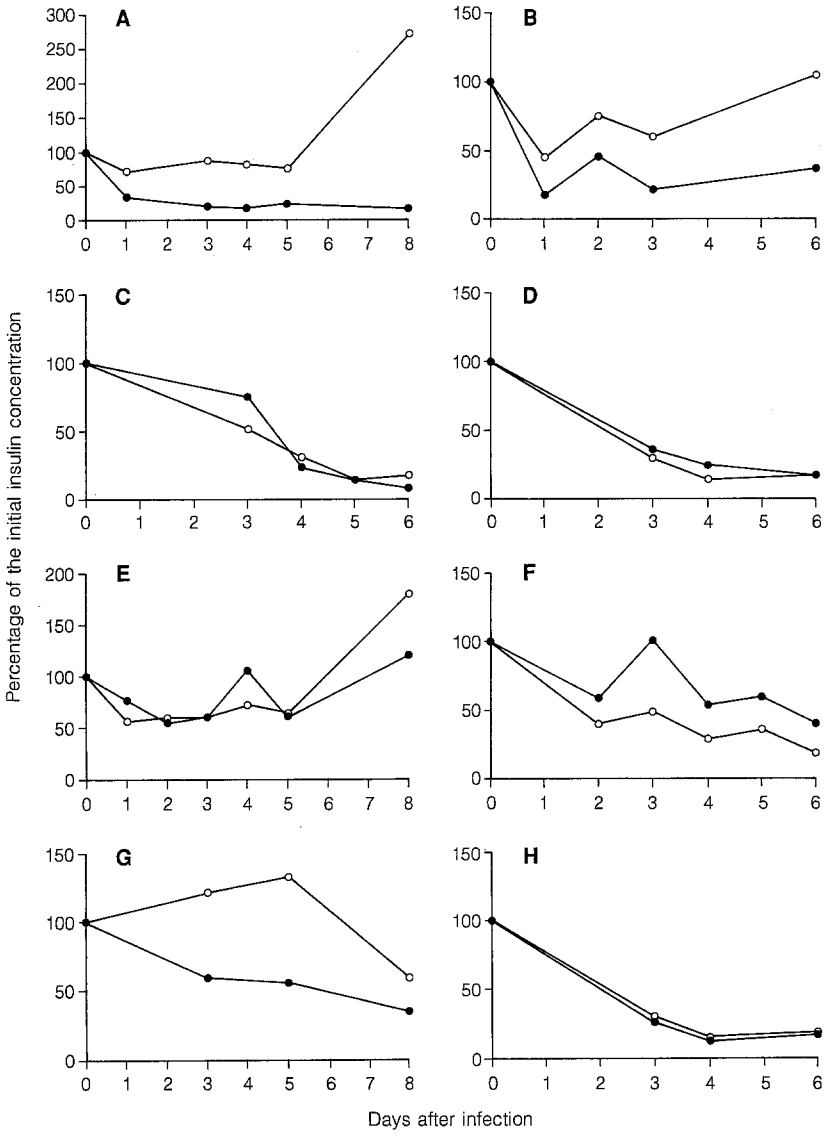
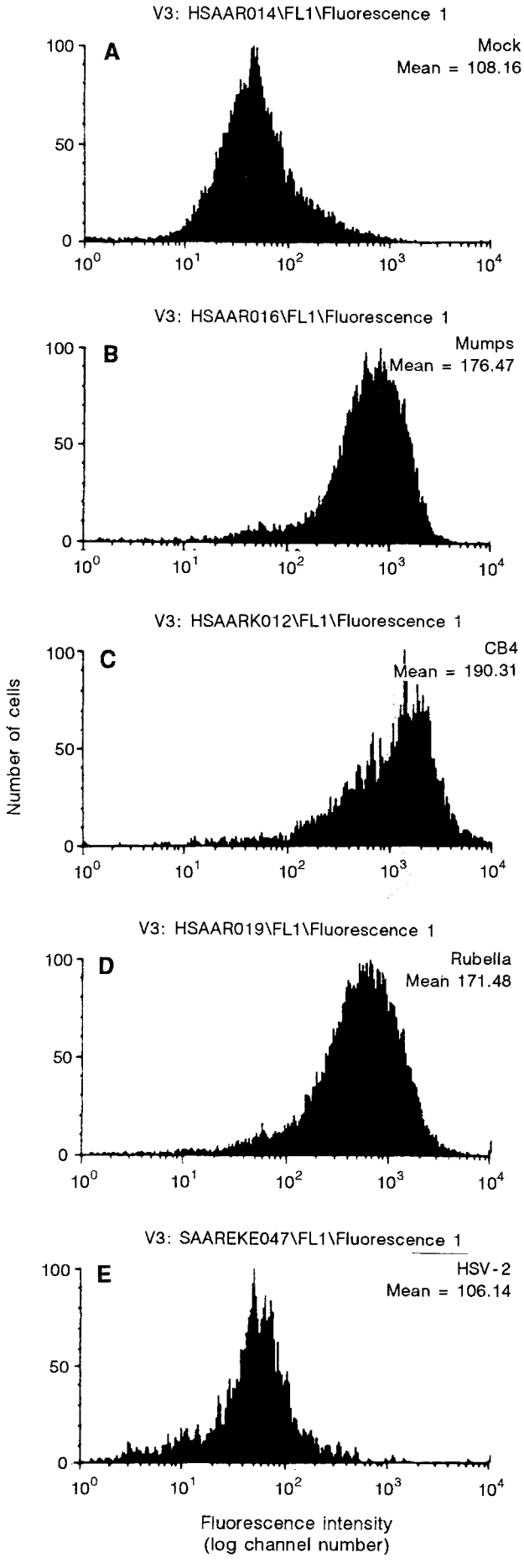


Fig. 2 A–H. Concentration of passively secreted insulin in culture mediums of mumps virus-infected and mock-infected islets in six different infection experiments (**A–F**). **G** shows insulin concentration in coxsackie B4 virus-infected and **H** in rubella virus-infected cultures. Each figure represents results from one experiment where virus-infected and non-infected cells were originating from the same pancreas. Culture supernatants were collected serially at different intervals after the infection. The x-axis represents days after the infection and y-axis the level of insulin concentration compared to the initial concentration at the beginning of the infection (%). Insulin concentration in mock-infected cultures is represented by open circles (○—○) and that in virus-infected cultures by filled circles (●—●)

remained lower than in control culture during the whole culture period (Fig. 2 A, B). This defect was most evident when insulin was allowed to concentrate into the medium for a few days. In four other experiments mumps infection had no clear effect on insulin secretion (Fig. 2 C–F) and no statistically significant difference was observed between mumps virus-infected and non-infected cultures. Insulin concentration was analysed also from one CB4 infected (Fig. 2 G) and one rubella virus-infected cultures (Fig. 2 H). In CB4-infected culture insulin concentration rapidly decreased after the infection while rubella virus infection had no effect on insulin concentration.

The effect of mumps virus on HLA expression was analysed in seven different islet cell cultures. In all these experiments mumps virus infection increased the expression of HLA class I molecules on islet cells. In mumps virus-infected cultures the average mean channel number representing the intensity of HLA class I staining in flow-cytometry was 722 (range 602–834) as compared with 548 (range 419–680) in uninfected control cultures ($p < 0.001$). A typical flow-cytometric histogram showing mumps virus-induced enhancement in class I expression is shown in Figure 3. Supernatants which were collected from infected islets and depleted from infective virus by ultraviolet irradiation (two experiments) were able to induce comparable class I expression in fibroblast cultures. The average increase in mean channel number was from 576 (medium from mock-infected islets) to 642 (medium from mumps virus-infected islets). Inactivated virus as such was not able to increase class I expression.

Without any outside given lymphokines only occasional islet cells expressed HLA-DR antigens and the expression did not change during mumps virus infection. However, IFN-gamma was a potent inducer of DR antigens in islet cells. The average IFN-gamma induced increase in mean channel number in flow-cytometry (four experiments) was from 416 (range 336–480) to 618 (range 615–622). Figure 4 shows the increase in the number of DR-positive cells from 2% to 44% in one typical experiment. In microscopic analyses none of the insulin cells were DR positive. When IFN-gamma was added to mumps virus-infected cultures a comparable increase in DR expression was found. However, in one of the four experiments mumps infection seemed to potentiate the ef-



fect of IFN-gamma (Fig. 4). TNF had no effect on DR-expression.

Rubella (two experiments) and CB4 viruses (four experiments) also induced a clear rise in the expression of class I molecules on islet cells (Fig. 3). In contrast, HSV-2 virus (two experiments) had no effect on the density of HLA molecules although the cells expressed viral proteins in immunofluorescence staining and infective virus could be isolated from culture supernatants throughout the culture period. None of these viruses caused any enhancement in DR expression.

Discussion

Hyperexpression of HLA class I molecules is one of the first signs of islet-cell pathology in Type 1 diabetes followed by aberrant expression of class II molecules on insulin-containing cells [12–14]. It has been suggested that these phenomena are among the primary pathogenetic events leading either to direct nonimmune impairment of Beta-cell function [14–17] or enhanced lymphocytotoxic immune response to Beta-cell autoantigens [18]. In the present study the possible role of viruses in aberrant HLA expression was analysed in virus-infected human fetal islet cell clusters which are formed by clustering of endocrine cells from collagenase-digested fetal pancreas tissue in standard cell culture conditions. It has been shown previously that 5–10% of these cells contain insulin being able to respond to physiological stimuli by insulin secretion [9, 19, 20]. Majority of the cells seem to be premature endocrine cells which are able to differentiate to e. g. Beta cells when transplanted to a living host [20].

In mumps virus-infected islets viral nucleocapsid antigen was found in 60% of cells, including Beta cells, and the virus could be isolated from the culture supernatants throughout the infection (6–8 days). These findings are in accordance with a previous study suggesting that mumps virus is able to replicate in Beta cells in vitro [7]. In spite of this, the infection seemed to have only a minor effect on insulin secretion.

The expression of HLA class I antigens clearly increased in mumps virus-infected islets whereas the expression of DR molecules was not enhanced. Inactivated virus was unable to cause class I induction suggesting that the presence of infective virus is necessary. Our results are in accordance with previous studies where infective reovirus was found to enhance the expression of class I molecules in Beta cells while inactivated virus was ineffective [18, 21]. In the present study also rubella and CB4 viruses enhanced the expression of class I but not class II mole-

Fig. 3 A–E. Flow-cytometric analysis of the density of HLA class I molecules on mock-infected and virus-infected islet cell preparations: mock (**A**), mumps (**B**), coxsackie B4 (CB4) (**C**), rubella (**D**) and herpes simplex virus 2 (HSV-2) (**E**) viruses. Fluorescence intensity on x-axis (channel number) represents the amount of bound anti-HLA class I antibody (green fluorescence, Fl1) and y-axis the number of cells per each channel. The mean fluorescence channel number is presented in each figure

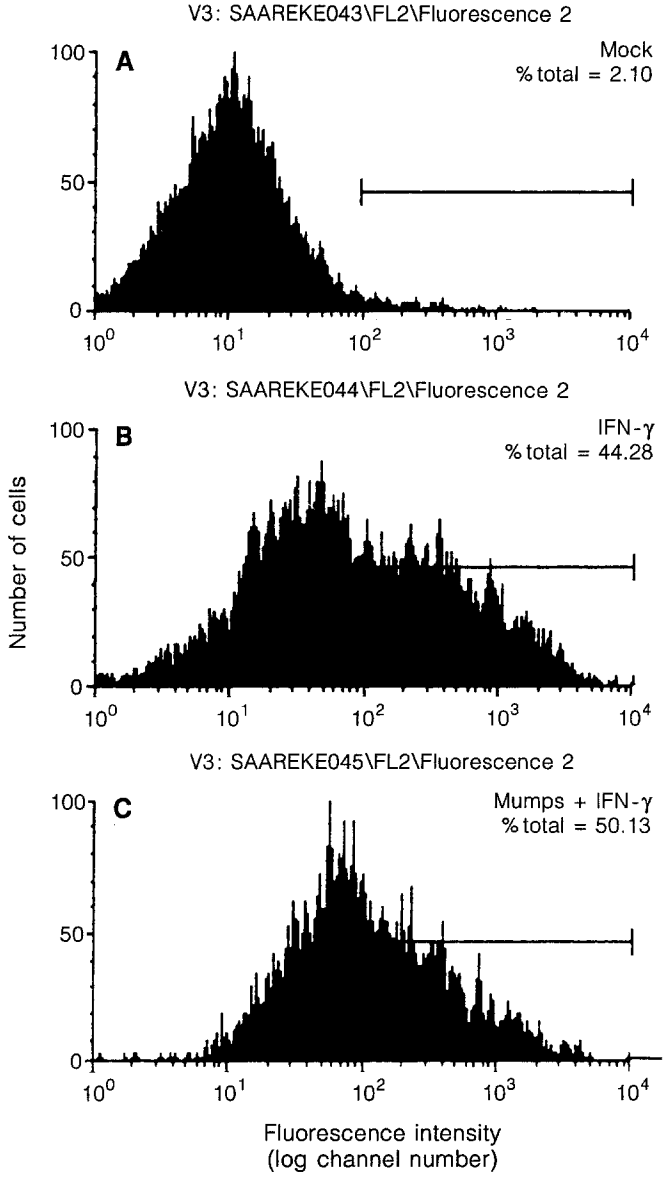


Fig. 4 A–C. Flow-cytometric analysis of the density 4 of HLA class II molecules on islet cells in untreated (**A**), and in cultures treated by interferon (IFN)-gamma alone (**B**) or by IFN-gamma together with mumps virus (**C**). x-axis represents the intensity of red fluorescence (mean channel number) reflecting the amount of bound anti-HLA class II antibody (red fluorescence, Fl2) and y-axis the number of cells per each channel. The percentage of DR positive cells is also presented in each figure

cules in islet cells. In flow-cytometry virus induced class I expression occurred in the whole islet cell population suggesting that this phenomenon appeared also in Beta cells.

The mechanism whereby virus infection induces the expression of HLA class I molecules is not known. Islet cell cultures were not contaminated by detectable amounts of T lymphocytes or monocytes indicating that the mechanism has to be independent from the function of these immunologically active cells. In theory, a direct effect of the virus on the regulation of HLA genes or indirect mechanisms mediated by factors synthesized by infected cells may be involved. This possibility was supported by the observation that ultraviolet-treated medium from mumps virus-infected islet cell cultures caused an increase in class I expression in fibroblasts comparable to that of the virus itself.

IFN-beta may play a major role as neutralizing antibodies to IFN-beta are able to block class I induction in mumps virus-infected fibroblasts [22]. This is in accordance with reports by King et al. [23] and Grundy et al. [24] who found that IFN-beta is an important mediator in enhanced class I expression induced by cytomegalo- and flaviviruses. IFN-alpha can also be induced during virus infections and it has been shown to be present in Beta cells hyperexpressing class I at the onset of diabetes [25].

Virus-induced class I expression may have general importance in the eradication of infection as it increases the activity of cytotoxic T cells [26]. However when occurring in islet cells this phenomenon may also increase the activity of autoreactive β-cell specific T lymphocytes. Interestingly, some viruses such as HSV and adenoviruses have no influence or may even decrease the expression of class I molecules on the cell surface [27–29] which also seemed to be the case in HSV-2 infected islet cells.

Beta cells are normally negative for class II antigens and they could not be induced to express DR molecules by mumps or any other viruses in fetal islet cell clusters.

This was not due to an inability of fetal cells to express DR molecules as indicated by IFN-gamma induction experiments. Our results are in accordance with previous studies suggesting that class II molecules can be induced on human islet cells by IFN-gamma [30–32]. However, Beta cells seem to remain class II negative which was also the case in the present study. This is in contrast to the situation in Type 1 diabetes where particularly insulin-containing cells express class II molecules [12, 33]. The significance of this discrepancy is not known but it suggests that other factors than IFN-gamma alone are important in aberrant DR expression during the diabetic process.

Results from our in vitro model suggest that viral infections may have profound effects on the immunological integrity of pancreatic Beta cells. They give support to the theory that viral infections may trigger the onset of Type 1 diabetes by directly disturbing the function of the cells and/or by stimulating autoaggressive immune response. Former mechanism may explain the observations of mumps virus-infections shortly before the onset of the disease [34] while the latter may explain the delayed temporal relationship observed in some other studies [35-38].

Acknowledgements. We thank Dr. M. Pelto-Huikko for very helpful instructions on immunofluorescence staining methods. Financial support by the Foundation for Diabetes Research in Finland, the Nordisk Insulin Fond and the Lehikoinen Foundation are also gratefully acknowledged.

References

1. Banatvala JE (1987) Insulin-dependent (juvenile-onset, type 1) diabetes mellitus – coxsackie B viruses revisited. Prog Med Virol 34: 33–54
2. Saggese G, Federico G, Garzelli C, Toniolo A (1985) Insulin-dependent diabetes: a possible viral disease. Pediatrician 12: 179–193

3. Yoon J-W, Onodera T, Notkins L (1978) Virus-induced diabetes mellitus. XV. Beta cell damage and insulin-dependent hyperglycemia in mice infected with coxsackie virus B4. J Exp Med 148: 1068–1080
4. Fujinami RS, Oldstone MBA (1989) Molecular mimicry as a mechanism for virus-induced autoimmunity. Immunol Res 8: 3–15
5. Toniola A, Federico G, Manocchio I, Onodera T (1988) Aetiology and pathogenesis of type 1 diabetes: viruses. In: Besser GM, Bodansky HJ, Cudworth AG (eds) Clinical Diabetes. Gower Medical Publishing, London, pp 10.1–10.20
6. Foulis AK (1989) In type 1 diabetes. Does a non-cytopathic viral infection of insulin-secreting B-cells initiate the disease process leading to their autoimmune destruction. Diab Med 6: 666–674
7. Prince GA, Jenson AB, Billups LC, Notkins AL (1978) Infection of human pancreatic beta cell cultures with mumps virus. Nature 271: 158–161
8. Yoon J-W, Onodera T, Jenson B, Notkins AL (1978) Virus-induced diabetes mellitus. Replication of coxsackie B3 virus in human pancreatic beta cell cultures. Diabetes 27: 778–781
9. Otonkoski T, Knip M, Panula P et al. (1988) Morphology, yield and functional integrity of islet-like cell clusters in tissue culture of human fetal pancreata obtained after different means of abortion. Acta Endocrinol 118: 68–76
10. Hellerström C, Lewis NJ, Borg H, Johnson R, Freinkel N (1979) Method for large-scale isolation of pancreatic islets by tissue culture of fetal rat pancreas. Diabetes 28: 769–776
11. Örvell C (1984) The reactions of monoclonal antibodies with structural proteins of mumps virus. J Immunol 132: 2622–2629
12. Bottazzo GF, Dean BM, McNally JM et al. (1985) In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. N Engl J Med 313: 353–360
13. Ono JS, Issa-Cherqui B, Colle E, Guttmann RD, Seemayer TA, Fuks A (1988) IDDM in rats – enhanced MHC class I heavy-chain gene expression in pancreatic islets. Diabetes 37: 1411–1418
14. Harrison LC, Campbell IL, Allison J, Miller JFAP (1989) Perspectives in diabetes. MHC molecules and beta-cell destruction. Immune and nonimmune mechanisms. Diabetes 38: 815–818
15. Allison J, Campbell IL, Morahan G, Mandel TE, Harrison LC, Miller JFAP (1988) Diabetes in transgenic mice resulting from over-expressing of class I histocompatibility molecules in pancreatic beta cells. Nature 333: 529–533
16. Sarvetnick N, Liggitt D, Pitts SL, Hansen SE, Stewart TA (1988) Insulin-dependent diabetes mellitus induced in transgenic mice by ectopic expression of class II MHC and interferon-gamma. Cell 52: 773–782
17. Lo D, Burkly LC, Widera G et al. (1988) Diabetes and tolerance in transgenic mice expressing class II MHC molecules in pancreatic beta cells. Cell 53: 159–168
18. Campbell IL, Harrison LC, Ashcroft RG, Jack I (1988) Reovirus infection enhances expression of class I MHC proteins on human beta cell and rat RINm5F cell. Diabetes 37: 362–365
19. Korsgren O, Sandler S, Landström AS, Jansson L, Andersson A (1988) Large-scale production of fetal porcine pancreatic islet-like cell clusters. An experimental tool for studies of islet cell differentiation and xenotransplantation. Transplantation 45: 509–514
20. Tuch BE, Grigoriou S, Turtle JR (1986) Growth and hormonal content of human fetal pancreas passaged in athymic mice. Diabetes 35: 464–469
21. Campbell IL, Harrison LC (1989) Viruses and cytokines: evidence for multiple roles in pancreatic beta cell destruction in type 1 insulin-dependent diabetes mellitus. J Cell Biochem 40: 57–66
22. Parkkonen P, Hyöty H, Koskinen L, Aaran R-K, Leinikki P (1990) Virus induced abnormalities in the function and phenotype of human pancreatic islet cells. In: Kenes (ed) The Proceedings of Immunology of Diabetes. Tel Aviv, p 17
23. King NJC, Kesson AM (1988) Interferon-independent increases in class I major histocompatibility complex antigen expression follow flavivirus infection. J Gen Virol 69: 2535–2543
24. Grundy JE, Ayles HM, McKeating JA, Butcher RG, Griffiths PD, Poulter LW (1988) Enhancement of class I HLA antigen expression by cytomegalovirus: role in amplification of virus infection. J Med Virol 25: 483–495
25. Foulis AK, Farquharson MA, Meanger A (1987) Immunoreactive alfa-interferon in insulin-secreting beta cells in type 1 diabetes mellitus. Lancet II: 1423–1427
26. Flyer DC, Burakoff SJ, Faller DV (1985) Retrovirus-induced changes in major histocompatibility complex antigen expression influence susceptibility to lysis by cytotoxic T lymphocytes. J Immunol 135: 2287–2292
27. Pääbo S, Nilsson T, Peterson PA (1986) Adenovirus of subgenera B, C, D und E modulate cell-surface expression of major histocompatibility complex class I antigens. Proc Natl Acad Sci USA 83: 9665–9669
28. Jennings SR, Rice PL, Kloszewski ED, Anderson RW, Thompson DL, Thevethia SS (1985) Effect of herpes simplex virus types 1 and 2 on surface expression of class I major histocompatibility complex antigens on infected cells. J Virol 56: 757–766
29. Pääbo S, Severinsson L, Andersson M, Martens I, Nilsson T, Peterson PA (1989) Adenovirus proteins and MHC expression. Adv Cancer Res 52: 151–163
30. Pujol-Borrell R, Todd I, Doshi M, Gray D, Feldmann M, Bottazzo GF (1986) Differential expression and regulation of MHC products in the endocrine and exocrine cells of the human pancreas. Clin Exp Immunol 65: 128–139
31. Campbell IL, Bizilj K, Colman PG, Tuch BE, Harrison LC (1986) Interferon-gamma induces the expression of HLA-A, B, C but not HLA-DR on human pancreatic beta cells. J Clin Endocrinol Metab 62: 1101–1109
32. Pujol-Borrell R, Todd I, Doshi M, Bottazzo GF, Sutton R, Gray D, Adolf GR, Feldman M (1987) HLA class II induction in human islet cells by interferon gamma plus tumor necrosis factor or lymphotoxin. Nature 326: 304–306
33. Foulis AK, Farquharson MA (1986) Aberrant expression of HLA-DR antigens by insulin-containing beta-cells in recent-onset type 1 diabetes mellitus. Diabetes 35: 1215–1224
34. Gamble DR (1980) Relation of antecedent illness to development of diabetes in children. Br Med J 281: 99–101
35. Hyöty H, Leinikki P, Reunanen A et al. (1988) Mumps infections in the etiology of type 1 (insulin-dependent) diabetes. Diabetes Res 9: 111–116
36. Gundersen E (1927) Is diabetes of infectious origin? J Inf Dis 41: 197–202
37. Melin K, Ursing B (1958) Diabetes mellitus som komplikation till parotis epidemica. Nord Med 27: 1715–1717
38. Sultz HA, Hart BA, Zielezny M, Schlesinger ER (1975) Is mumps virus an etiologic factor in juvenile diabetes mellitus? J Pediatr 86: 654–656

Received: 7 June 1991
and in revised form: 9 September 1991

Dr. H. Hyöty
Institute of Biomedical Sciences
University of Tampere
P. O. Box 607
SF-33101 Tampere
Finland