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Enhanced expression of MHC class I molecules on cultured human thyroid follicular cells infected with reovirus through induction of type 1 interferons

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SUMMARY

Certain viruses are known to modulate the cellular expression of MHC molecules. We have investigated whether reovirus types 1 or 3 can alter the normal MHC molecule expression on cultured human thyroid follicular cells (TFC). Primary TFC cultures were established from eight human thyroid donors and MHC class I and II expression was assessed by indirect immunofluorescence microscopy. Both types of reovirus enhanced MHC class I expression on TFC from all thyroid donors. Class II MHC protein was strongly induced by type 1 reovirus on TFC from one donor, while weak induction of expression, by either reo-1 or reo-3 virus, was noted on the TFC of five other donors. Studies on the mechanism(s) of MHC class I hyperexpression showed that mouse MoAb against the type 3 reovirus haemagglutinin (anti-HA3) reduced the ability of the virus to induce hyperexpression of class I MHC molecules on TFC. However, supernatant harvested from type 3 reovirus-infected TFC cultures maintained its ability to enhance class I expression after incubation with anti-HA3. Moreover, adding rabbit anti-sera to interferon-alpha (IFN-α) or IFN-β inhibited the increased class I MHC expression on TFC by both types of reovirus. These data suggest that reoviruses (types 1 and 3) can enhance MHC class I on cultured TFC. The mechanism of MHC class I enhancement is most probably through the release of IFN-α and IFN-β.

Keywords reovirus MHC molecules thyroid follicular cells IFN-α IFN-β

INTRODUCTION

The class I and class II molecules of the MHC are integral to the initiation (class II) and effector (classes I and II) functions of the immune response [1]. Class I MHC proteins are normally expressed on all nucleated cells, whilst class II expression is mainly restricted to antigen-presenting cells such as monocytes, lymphocytes and dendritic cells under normal circumstances. Hyperexpression of MHC class I molecules by target parenchymal cells has been demonstrated in a variety of autoimmune conditions, e.g. β cells of the pancreas in patients with type 1 diabetes mellitus and on thyrocytes in autoimmune thyroid diseases [2–4]. Aberrant expression of MHC class II molecules has been described on thyrocytes from patients with autoimmune thyroid diseases [4] and on β cells of the pancreas in patients with type 1 diabetes mellitus [3]. These findings are consistent with the hypothesis that dysregulation of MHC molecule expression by target cells may be an initiating or propagating factor in autoimmune responses by themselves presenting self constituents to autoreactive helper T cells [5].

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It has also been suggested that a local viral infection may cause the production of cytokines such as interferon-gamma (IFN-γ) which in turn induce MHC class II expression.

Reoviruses are double-stranded RNA viruses divided into three major serotypes, 1, 2 and 3 [6]. Types 1 and 3 reoviruses (reo-1 and reo-3 viruses) are notable for their association with autoimmune diseases in mice, including diabetes mellitus and thryoid autoimmune disease [7–10]. Human disease following reovirus infection is rare, although the virus is highly prevalent, as determined by antibody survey [11]. Enteritis and upper respiratory tract infection have been reported in infants and children, and adult volunteers have developed similar syndromes [11]. It has also been shown that *in vitro* infections of rat and mice thyroid cell lines with reo-1 and reo-3 viruses can induce MHC class II expression [12,13]. In one study [12] the induction of class II was attributed to direct binding of the virus, as class II expression was also shown by inactivated virus particles, while in the other [13] it was not established whether class II-expressing cells were infected with the virus. The effect of reovirus on MHC expression by human thyroid cells has not been studied previously.

The study reported here was designed to investigate whether infection of human thyroid follicular cell (TFC) cultures with

reoviruses is associated with alteration in the expression of class I and II MHC molecules. We also investigated whether these were direct viral effects or cytokine-mediated.

MATERIALS AND METHODS

Thryoid tissues and TFC cultures

Fresh thyroid tissue was obtained from patients whose thyroids were removed for reasons other than thyroid autoimmune disease, namely laryngeal carcinoma in six (HTN 9, 10, 13, 18, 19 and 30) and diffuse goitre in two (HTN 38, 39). The thyroid specimens were finely minced with scissors within 1–2 h of removal, extensively washed in Hanks' balanced salt solution (HBSS; Sigma, Poole, UK) and digested with 1 mg/ml porcine collagenase (Lorne Laboratories, Ltd., Reading, UK) in HBSS at 37°C for 1–2 h. Supernatants of the digests were collected and washed three times in HBSS. Subsequently the cells were treated with 0·83% ammonium chloride solution for 10 min to lyse contaminating erythrocytes, then washed three times in HBSS. Cell viability after dispersion was assessed using 0·15% trypan blue as a differential stain. Approximate cell counts only were possible owing to the presence of some undisrupted follicles. Thyroid cells were then either grown in tissue culture flasks or stored frozen in liquid nitrogen after slow cooling in 10% dimethyl sulphoxide, 20% fetal calf serum (FCS) in RPMI 1640 medium (Imperial Laboratories).

Freshly prepared or thawed TFC were grown first in 75-cm^2 tissue culture flasks (Costar, Cambridge, MA) at 37°C in 5% CO_2, and maintained in 10% FCS in thyrocyte growth medium (TGM; pH 7·2) which consisted of RPMI 1640 medium containing 2 mM L-glutamine (Imperial Laboratories), 10 mM HEPES (BDH, Poole, UK), 100 U/ml penicillin and 100 μg/ml streptomycin (Imperial Laboratories), 10 μg/ml insulin (Wellcome), 10 ng/ml glycyl-l-histidyl-l-lysine acetate (Sigma), 10 nM hydrocortisone (Sigma), 5 μg/ml transferrin (Sigma) and 10 mU/ml bovine thyroid-stimulating hormone (Sigma). When cells reached confluence, as assessed by phase contrast microscopy, they were removed from the flasks with 0·25% trypsin (Difco, Detroit, MI) in PBS pH 7·2, containing 0·3 mg/ml EDTA, then washed three times in the RPMI 1640 medium. Aliquots of 500 μl of thyrocytes ($0{\cdot}2 \times 10^6$/ml) in TGM were added to 13-mm diameter cover slips placed in wells of 24-well tissue culture plates (Costar). The cells were allowed to grow on the coverslips for 2–3 days before infection with the reoviruses.

Identification of TFC in primary cultures

Thyroid cells were tested for cytokeratin, which is present in epithelial cells but not fibroblasts, by indirect immunofluorescence staining using a MoAb directed against cytokeratin peptide 8 (Sigma). More than 95% of the cells showed positive staining for cytokeratin. Consistent results were obtained when TFC were identified by indirect immunofluorescence staining using human sera containing high titres of anti-thyroglobulin autoantibodies.

Reovirus infection

Reo-1 and reo-3 viruses were grown in Vero cells (monkey kidney) in 75-cm^2 flasks until the cell monolayer demonstrated a 100% cytopathic effect (CPE, after 6 days). Tissue culture supernatant was decanted, centrifuged at 2000 *g* for 5 min to clear the cellular debris, aliquotted and stored at −70°C. The viral titre in the culture supernatant was determined by inoculation of a replicate tube of Vero cells with serial 10-fold dilutions of supernatant and observing the development of CPE over a period of 10 days ($TCID_{50}$ assay: standard tissue culture infectious dose). The dose of the virus used in subsequent experiments was 10–100-fold $TCID_{50}$, which was shown in preliminary experiments to ensure viral infection of thyroid cultures, whilst not resulting in significant cell death within the time period of the experiment (4 days). Supernatant of mock-infected Vero cells, processed exactly as above, was used as negative control.

Phytohaemagglutinin and interferon treatment

Phytohaemagglutinin (PHA; Sigma) at 10 μg/ml in TGM was added to parallel uninfected TFC cultures and used as positive control to induce ectopic expression of MHC class II and to enhance class I expression. IFN-α (Wellferon; Wellcome) at 1000 U/ml and IFN-β (Sigma) at 400 U/ml were also incubated on parallel uninfected cultures to serve as positive control for MHC class I expression (the dose of IFN was determined as described below).

Depletion of reoviruses

Reo-1 and -3 viruses were depleted from supernatants harvested from virus-infected TFC cultures in two steps. Initially these supernatants were subjected to ultracentrifugation at 100 000 *g* for 4 h. The supernatants were then incubated for 1 h at 4°C on Vero cell monolayers to adsorb the remaining virus particles. The adsorption process was repeated a further two times. The removal of the virus was verified by the absence of CPE on Vero cell monolayers 2 weeks after the addition of the virus-depleted supernatant.

Neutralization of reo-3 virus

Purified mouse IgG MoAb to reo-3 virus haemagglutinin (σ1) (anti-HA3, provided by Dr H. U. Saragovi, University of Pennysylvania Medical School, Philadelphia, PA) was used to inhibit the binding of reo-3 virus to its target cells. The amount of anti-HA3 required to neutralize reo-3 virus was determined by incubating the virus with increasing concentrations of anti-HA3 for 1 h at 37°C. The mixtures were then incubated on replicate tubes of Vero cell monolayers, and monitored for CPE over 14 days of incubation. Anti-HA3 at 100 μg/ml was the minimum concentration required to inhibit the CPE of the virus. Therefore, reo-3 virus or supernatant from reo-3 virus-infected TFC culture was incubated with anti-HA3 at 100 μg/ml antibody for 1 h at 37°C. The mixtures were then added to parallel cultures of TFC.

Neutralization of interferon activity

IFN-α and IFN-β are known to enhance class I expression on TFC. The release of these cytokines from virus-infected thyroid cell preparation was examined by an inhibition assay. In this assay the presence of these cytokines was tested by inhibiting hyperexpression of class I molecules using specific antibodies (i.e. anti-IFN-α and anti-IFN-β). Initially we determined the concentration of exogenous cytokines required to cause maximum increase in class I expression on TFC, then the amount of the specific antibodies required to inhibit the action of IFN-α and IFN-β was estimated.

IFN-α (Wellcome) and IFN-β (Sigma) at concentrations of 200, 400, 800, 1000 and 2000 U/ml were added to replicate of TFC cultures. After 4 days of incubation at 37°C, the level of class I expression was examined by immunofluorescence staining. IFN-α at 1000 U/ml and IFN-β at 400 U/ml were found to induce the highest level of class I expression on TFC.

Aliquots of sheep anti-IFN-α antibodies (ICN, Thame, UK) at 20, 40, 80, 100 and 200 μg/ml TGM were incubated with IFN-α at 1000 U/ml for 1 h at 37°C. In similar conditions, aliquots of sheep anti-IFN-β antibodies (Lee Biomolecular Research Inc, San Diego, CA) at 20, 40, 80, 100 and 200 U/ml TGM were incubated with IFN-β at 400 U/ml. Normal rabbit serum was also added to the cytokines to serve as a control for non-specific inhibitor that might occur in the rabbit sera. Subsequently these mixtures were added to replicate of thyroid cultures, and class I expression was determined by immunofluorescence microscopy 4 days later. Concentrations of anti-IFN-α antibodies at 400 μg/ml and anti-IFN-β at 200 U/ml were sufficient to reverse the hyper-expression of class I molecules by IFN-α and IFN-β, respectively. These antibodies did not show cross-reaction, i.e. antibodies against one cytokine did not inhibit the action of the other cytokine. Normal rabbit serum did not inhibit the action of IFN-α or IFN-β on class I expression.

To test the release of IFN-α and IFN-β from reovirus-infected thyroid cell preparation, anti-IFN-α at 400 μg/ml or anti-IFN-β at 200 U/ml were added to reo-1 or reo-3 virus-infected TFC cultures. After 4 days of incubation class I expression was assessed by immunofluorescence microscopy. Replicate TFC cultures were also incubated with IFN-α at 400 U/ml or IFN-β at 200 U/ml, with or without the inhibiting dose of the antibodies, to serve as control for the action of the cytokines and the antibodies.

Detection of MHC proteins

Indirect immunofluorescence staining was used for detection of MHC protein expression on cultured thyrocytes as previously described by Pujol-Borrell *et al.* [14]. Briefly, at the end of incubation with the virus the cover slips were washed three times in the RPMI 1640 medium. All of the following staining steps were carried out at room temperature in a humid chamber. For cytoplasmic staining the cells were prefixed with acetone : methanol (1 : 1) by 5 min incubation. The cover-slips were then washed by rinsing once in 0·2% bovine serum albumin (BSA) in HBSS and twice in HBSS alone. Mouse IgG2a MoAb directed to human HLA-A, B and C at 1 : 50 in PBS (HLA-ABC, W6/32; Dako) or mouse IgG2a MoAb to HLA-DR at 1 : 50 in PBS (HLA-DR, CR2/43; Dako) were added to cover slips. After 30 min the cover slips were washed as described above, then FITC-conjugated goat anti-mouse IgG (Sigma) was added to all cover slips. Following a further 30 min incubation the cover slips were washed three times as described above and once with PBS. The coverslips were mounted on microscope slides and examined by fluorescence microscopy (Leitz). For surface staining the cells were fixed with 5% acetic acid in ethanol for 10 min at −20°C, after the staining procedure, instead of the initial fixation with methanol : acetone.

As a control for non-specific binding of the FITC conjugate to TFC, coverslips were included with PBS, instead of the MoAb. This was followed by the addition of FITC anti-mouse IgG conjugate as described above.

The levels of MHC class I and II expression on TFC infected with the viruses were compared with that of negative control TFC cultures (incubated with supernatant of virus-negative Vero cells) and positive control TFC cultures (stimulated by PHA or IFN). The expression was assessed by fluorescence microscopy and scored according to the scoring system of Todd *et al.* [15] (see footnote to Table 1).

Table 1. The effect of reovirus infection on MHC class I and class II expression on cultured thyroid follicular cells (TFC) from eight thyroid donors*

	MHC class I		MHC class II	
Thyroid specimen	Reo-1	Reo-3	Reo-1	Reo-3
HTN 9	↑↑	↑	±	+
HTN 10	↑↑	↑↑	−	−
HTN 13	↑↑	↑	+	−
HTN 18	↑↑	↑	++	±
HTN 19	↑↑	↑↑	+	±
HTN 30	↑↑	↑↑	−	−
HTN 38	↑↑	↑↑	±	±
HTN 39	↑↑	↑↑	+	+

* MHC class I and class II expression was assessed by fluorescence microscopy, and the scoring system of Todd *et al.* [15] was adapted as follows: for class I (HLA-A, B and C), → = basal positive seen on unstimulated thyrocytes, ↑ = more than 40% significant enhancement over basal level, ↑↑ = more than 80% very strongly enhanced over basal level. For HLA DR: − = no positive cells, ± = more than 10% positive or 10–30% weak positive, + = 10–30% positive or 30–80% weak positive, ++ = 30–80% positive, or more than 80% weak positive, + + + = more than 80% positive.

RESULTS

The effect of reoviruses on MHC expression

Enhanced expression of MHC class I proteins was first noted 3 days after infection with either reo-1 or reo-3 virus. From day 8 of infection the expression of class I MHC molecules started to decrease, and reached the negative control level by day 14. Consequently 4-day incubations were carried out during the study.

The up-regulation of MHC class I expression was induced by reo-1 and reo-3 viruses on TFC from all donors (Table 1). Such an increase of MHC class I expression was demonstrable by both cytoplasmic and surface staining of the TFC. This was repeated with similar results on TFC.

TFC from the eight donors were also stained for HLA-DR. Strong expression of class II was induced by type 1 reovirus on TFC from one donor only (Table 1). Five other donors showed weak expression of class II proteins on TFC following infection by either reo-1 or reo-3 virus.

Mechanism of enhancement of MHC class I expression

The mechanism of MHC class I hyperexpression by reoviruses was investigated using the following methods.

The effect of depletion of the viruses from virus-infected TFC culture supernatants on MHC class I expression. To determine whether MHC class I hyperexpression was a consequence of the release of soluble cytokines by the virus-infected TFC supernatant fluids were collected from virus-infected TFC cultures, cleared of virus, and added to non-infected TFC cultures. The ability of virus-depleted TFC supernatant to increase MHC class I expression on TFC was compared with that of supernatant not depleted of virus. The virus non-depleted supernatants from reo-1 and reo-3 virus-infected thyrocytes caused significant increase in MHC class I expression equivalent to that caused by direct infection of TFC with either type of reovirus. When the TFC culture supernatants were depleted of the viruses (by ultracentrifugation followed by adsorption on Vero cell monolayers), no significant increase in expression of MHC class I was noted on TFC (Table 2a). However, the adsorption of the supernatants with Vero cells might remove other factors in addition to virus (e.g. cytokines).

The effects of blocking reo-3 virus binding to target cells. The effect of blocking reo-3 virus binding to target cells on the expression of class I molecules was also examined. The dose of anti-HA3 MoAb (which blocks reo-3 virus haemagglutinin) that can inhibit CPE on Vero cells, was found to inhibit the reo-3 virus from enhancing class I MHC molecule expression on TFC. However, supernatant from reo-3 virus-infected TFC cultures incubated with the anti-HA3 MoAb maintained its ability to enhance MHC class I expression on TFC from the same thyroid donor (Table 2c). This suggested that, in addition to the virus, other factors in the TFC supernatant could enhance class I expression.

Table 2. Studies of the mechanisms of MHC class I enhancement by reovirus on thyroid follicular cells (TFC) from one thyroid donor (HTN38).

Experimental condition	MHC class I expression
a. Control	
Negative control*	→
IFN-α	↑↑
IFN-β	↑↑
Reo-3 virus	↑↑
Reo-1 virus	↑↑
b. Effect of removal of the virus from the Sn of infected TFC cultures	
Sn from TFC culture infected with reo-1 virus	↑↑
Reo-1 removed from the Sn	→
Sn from TFC culture infected with reo-3 virus	↑↑
Reo-3 removed from the Sn	→
c. Effect of neutralizing reo-3 virus	
Reo-3 preincubated with anti-HA3	→
Sn from reo-3 virus-infected TFC preincubated with anti-HA3	↑
d. Effect of neutralizing interferon	
Reo-1 virus with antibodies to IFN-α	↑
Reo-1 virus with antibodies to IFN-β	→
Reo-3 virus with antibodies to IFN-α	↑
Reo-3 virus with antibodies to IFN-β	→
Reo-1 virus with antibodies to IFN-α and antibodies to IFN-β	→
Reo-3 virus with antibodies to IFN-α and antibodies to IFN-β	→

* Negative control, TFC incubated with virus negative supernatant of vero cell monolayer; Sn, supernatant from TFC infected with either of the reoviruses; anti-HA3, mouse MoAb to reo-3 virus haemagglutinin.

The effects of neutralization of interferon activity. IFN-α and IFN-β are known to enhance MHC class I expression on TFC. The release of these cytokines in TFC cultures infected with reovirus was indirectly detected by inhibiting their action by specific antibodies. Rabbit anti-human IFN-β antibodies reduced the capacity of reo-1 and reo-3 viruses to enhance MHC class I expression. Rabbit anti-human IFN-α antibodies were less potent in reducing MHC class I enhancement by the reoviruses (Table 2d).

DISCUSSION

Previous studies have shown that reo-1 and reo-3 viruses can enhance MHC class I expression on human β cells of the pancreas and can induce MHC class II on murine thyrocytes [12,13,16]. The findings presented in this study demonstrate that reo-1 and reo-3 viruses can enhance the expression of MHC class I molecules on human thyroid cells. This marked increase in MHC class I expression was readily demonstrated by fluorescence microscopy in eight out of eight thyroid specimens studied. However, the ability of reoviruses to induce class II molecules on TFC was not a prominent finding apart from TFC from 1/8 thyroid donors. At least 40–80% of cells in the thyroid preparations showed enhanced MHC expression in the reovirus-infected cultures: it is most likely that these cells are TFC, since more than 95% of the cells in the thyroid preparations are TFC as judged by anti-cytokeratin and anti-thyroglobulin staining.

Although reoviruses are known to be potent inducers of interferon [17,18], recent studies have suggested a direct viral role in the modulation of MHC expression [12,16]. Our data indicate that cytokines released after infection with reoviruses contribute to MHC class I hyperexpression of human TFC. Blocking the reo-3 viral haemagglutinin by anti-HA3 MoAb, before infection of TFC, inhibited the induction of MHC class I hyperexpression on TFC, whereas supernatants collected from reo-3 virus-infected TFC cultures and incubated with anti-HA3 maintained their ability to enhance MHC class I expression. In both cases the MoAb could have inhibited the binding of reo-3 virus to its target cells, but the preservation of the supernatant's ability to increase class I expression indicated that factors other than the virus caused the increased class I expression. Ultracentrifugation and virus adsorption were efficient in removal of virus from supernatants collected from virus-infected TFC cultures and inhibited the ability of the supernatants to enhance class I expression. However, cytokine levels and activity could also be affected by such treatment. Therefore, the possibility of a cytokine effect was further examined. Confirmatory evidence for cytokine-mediated effects was the ability of antibodies against IFN-α or IFN-β to inhibit the viruses' enhancement of MHC class I expression.

IFN-β played a major role in the hyperexpression of MHC class I by the reovirus-infected TFC. After selective depletion of IFN-β, IFN-α present in the supernatant of reovirus-infected TFC cultures was unable to maintain the hyperexpression of

MHC class I molecules, while after depletion of IFN-α the hyperexpression of class I was maintained by the action of IFN-β, which is also present in the supernatant. Depletion of both cytokines by adding anti-IFN-α and IFN-β simultaneously to the cultures, inhibited increased class I expression by the TFC. Amongst other cells, IFN-α is known to be produced by leucocytes and IFN-β by fibroblasts, and the presence of small numbers of such cells can not be excluded. It is also unclear whether these cells or the thyrocytes are the sources of the cytokines, and which of these cells are infected with the virus deserves further investigation.

The low and variable ability of reovirus to induce MHC class II expression by TFC could be a direct virus effect, but may also be explained by cytokine production. MHC class II is normally induced by IFN-γ, but not by IFN-α or IFN-β [15]. IFN-γ is produced by activated lymphocytes, therefore the presence of virus-specific T cells is required for the production of IFN-γ. However, the presence of reovirus-specific T cells which secrete IFN-γ may vary among the different thyroid donors, and this may explain the variable expression of MHC class II in this study. Moreover the action of IFN-γ may have been suppressed by the coexistence of type 1 IFN. It has been shown previously that IFN-α 1 can suppress class II induction on thyrocytes by IFN-γ [19]. IFN-β has also been found to antagonize IFN-γ-induced expression of Ia antigen on murine macrophages [20].

A possible role of viral infection of target cells in autoimmune pathogenesis is indirectly suggested in both human and animal insulin-dependent diabetes mellitus (IDDM). Indeed, the histological studies by Foulis *et al.* [21] of pancreas from recent-onset IDDM patients suggest a scenario similar to the experimental reovirus/TFC system described here. Thus, their data suggest that enhanced HLA class I expression by islet cells is an early pathogenic event (preceding HLA class II expression by islet β cells) [3] and this is associated with expression of IFN-α by the β cells [21]. Furthermore, in the BB rat model of type I diabetes, islet expression of IFN-α precedes lymphocytic infiltration and diabetes [22].

The regulation of MHC gene products by viruses (directly or indirectly) represents a significant immunological phenomenon because of the central role of MHC molecules in antigen presentation. 'Non-professional' antigen-presenting cells (APC) such as TFC may have antigen-processing machinery different from that of 'professional' APC, e.g. macrophages. Therefore, such non-professional APC may present novel autoantigen peptides that have not previously been experienced by thymic dendritic cells [23]. These peptides of self antigens would then be considered as foreign, and an autoimmune response might be generated against them.

Many studies have shown that target cells with dysregulated MHC molecules can stimulate T cells. TFC from autoimmune thyroid diseased glands have been shown to be capable of presenting autoantigens to self-reactive lymphocytes and to enhance the activation of autologous lymphocytes [24–26]. Additionally, it has been demonstrated that susceptibility to killing by cytotoxic T cells is dependent on the level of MHC class I expression by the target cell [27]. This may suggest that reovirus-infected TFC with increased MHC class I expression are more susceptible to cytotoxic T cells.

The abnormal expression of class I or class II antigens on target cells *per se* might not be sufficient to induce T lymphocyte activation, and other signals or factors might be required to initiate an autoimmune attack on target cells. TFC have demonstrated the capacity to express intercellular adhesion molecule-1 (ICAM-1), which is responsible for enhanced T cell binding to TFC [28]. However, two other groups failed to detect ICAM-1 on TFC from patients with Graves' disease [29,30]. Therefore the role of ICAM-1 in providing a second signal could not be confirmed. B7 is a major costimulatory signal in T cell activation, but these molecules could not be detected on TFC, and they were not induced on these cells in response to IFN-γ [31]. However, treatment of TFC with phorbol esters, which activate protein kinase C, results in consistent enhancement of the ability of the TFC cultures to stimulate T cell alloreactivity [32].

We would suggest that the reovirus-induced MHC class I hyperexpression by TFC may contribute to the generation of primed autoreactive T cells which can be stimulated by TFC presenting autoantigens since they are less dependent on co-stimulatory signals (Fig. 1). The release of type-1 IFN by the virus-infected thyrocytes will increase their class I

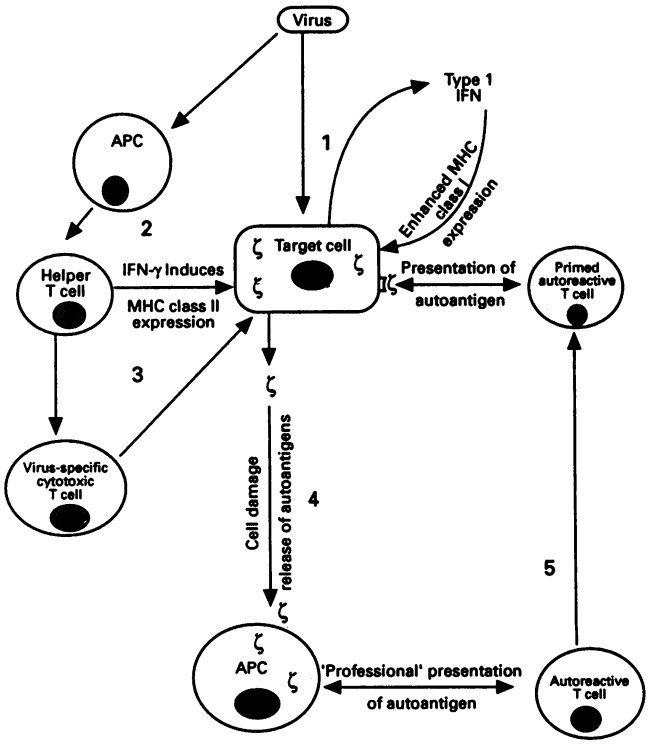

Fig. 1. The possible role of viruses in autoimmune thyroid disease. (1) Viral infection of thyrocytes (target cells) may lead to the release of type-1 interferons (IFN) which enhance MHC class I expression. (2) The virus may also be presented by 'professional' antigen-presenting cells (APC) to helper T cells, which can then stimulate virus-specific cytotoxic T cells, and also stimulate MHC class II expression on thyrocytes by secretion of IFN-γ. (3) The cytotoxic T cells will attack the thyrocytes presenting the virus peptides and hyperexpressing class I molecules. (4) This may lead to the release of thyrocyte autoantigens (ξ) which may be presented by 'professional' APC and lead to the activation of naive autoreactive T cells. (5) This will generate primed autoreactive T cells, which are less dependent on co-stimulatory signals and can therefore be activated by autoantigens presented by MHC molecules on the thyrocytes.

expression. Infection with the virus will also lead to the presentation of the virus peptides by professional APC to the helper T cells, which can then stimulate virus-specific cytotoxic T cells. The helper T cells will also secrete IFN-γ, which can then stimulate MHC class II expression. The cytotoxic T cells will then attack the virus-infected thyrocytes with enhanced class I expression, leading to the release of autoantigens. Presentation of such autoantigens by 'professional' APC will activate autoreactive T cells. This will generate primed autoreactive T cells, which are less dependent on co-stimulatory signals and can therefore be activated by autoantigen presented by MHC class II molecules on thyrocytes.

In conclusion, reoviruses consistently enhanced the expression of MHC class I molecules on cultured human TFC. There was a low and variable expression of class II molecules on TFC after reovirus infection. The mechanism of enhancement of MHC class I expression seems to be mediated by cytokines released from virus-infected cells.

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