American Journal of Pathology, Vol. 138, No. 5, May 1991

Induction of HLA-DR Expression on Thyroid Follicular Cells by Cytomegalovirus Infection *In Vitro*

Evidence for a Dual Mechanism of Induction

Emilio L. Khoury,* Lenore Pereira,* and Francis S. Greenspan†

From the Divisions of Oral Biology and Endocrinology,† University of California at San Francisco, San Francisco, California*

Cytomegalovirus (CMV) infection of primary cultures established from human thyroid nodular and normal (paranodular) tissues resulted in induction of human leukocyte antigen (HLA) DR expression on thyroid follicular cells (TFC), as detected by cell-surface immunofluorescence staining with monoclonal antibodies (MAb). Two distinct modalities of induction were observed. The first type occurred in cultures of normal tissue obtained from CMV-seropositive but not seronegative donors, was detected on 30% to 50% of the TFCs, even though the vast majority of these cells failed to show any morphologic or antigenic evidence of individual CMV infection, and was associated with production of γ-interferon (γ-IFN) in vitro. ***The induced molecules displayed the characteristic DR polypeptide profile on immunoprecipitation and electrophoretic analysis. These results demonstrate that CMV infection of normal thyroid cultures may induce DR expression on TFCs in the absence of pre-existing lymphoid infiltrates and suggest that the induction is the result of an*** in vitro ***response to CMV by previously sensitized immunocompetent cells present in these primary cultures. Such a response, associated with the release of γ-IFN, would induce DR expression on neighboring uninfected cells. The second mode of induction occurred in all CMV-infected cultures, regardless of their tissue origin (nodular or normal) or the serologic status of the donors. Up to 50% of infected TFCs at a late stage of infection, having fully developed CMV antigen-positive intranuclear inclusions, also displayed the cell-surface DR-related determinant recognized by one of the four anti-DR MAbs used. This induction was restricted to TFCs, while CMV-infected fibroblastoid cells present in the monolayers were invariably negative. Induction by CMV of major histocompatibility class II antigens on human epithelial cells may have significant implications in the development of normal immune responses against local viral infection, the enhancement of alloimmune rejection of grafted organs, and the generation of organ-specific autoimmune responses. (Am J Pathol 1991, 138:1209–1223)***

The class II human leukocyte antigens (HLA) are polymorphic cell-surface glycoproteins encoded by the D region of the major histocompatibility complex (MHC) genetic locus. They play a crucial role in the generation and regulation of adaptive immune responses to foreign (eg, viral) agents due to their ability to bind antigenic peptides derived from these agents and to serve as restriction elements for the recognition by, and activation of, autologous $CD4^+$ T lymphocytes with specific receptors for those epitopes.[1] Spontaneous expression of class II HLA antigens in nonlymphoid tissues was initially considered to be restricted to vascular endothelium and those myeloid-derived cells (macrophages, dendritic cells) normally engaged in antigen presentation to T lymphocytes. However it has been established that certain epithelial and endocrine cell types also are able to synthesize and express class II molecules.[2,3] Thyroid follicular cells (TFC) of normal glands do not express class II antigens *in vivo*[4] or *in vitro*[5] and contain only low levels of the corresponding mRNAs.[6] However cultured TFCs can be induced readily to express HLA-DR molecules with

This work was supported by NIH Grant DK36164. Presented in part at the International Workshop on Autoimmune Thyroiditis, University of Homburg (Saar), Federal Republic of Germany, October 11, 1989.

Accepted for publication January 7, 1991.

Address reprint requests to Emilio L. Khoury, MD, Department of Medicine, University of California San Francisco, 1710 Scott St., Third Floor, San Francisco, CA 94115.

γ-interferon [γ-IFN][7,8] or mitogenic lectins,[5] the latter probably acting through the local release of γ-IFN by residual lymphocytes present in the primary thyroid cultures.[8,9] This inducibility, shared with many other cell types,[10] is reflected *in vivo* by the TFC ectopic DR expression found in glands affected by ongoing cell-mediated immune phenomena.[4] These observations suggest the possibility that inducible DR expression enables TFCs to behave as antigen-presenting cells and thus to participate directly in the generation of specific immune responses to local infections. It has also been proposed[11] that DR antigens would confer on TFCs a similar ability to present their own cellular constituents to self-reactive $CD4^+$ T lymphocytes and trigger organ-specific autoimmunity.

There is some indication that viruses may indeed play a role in this inducible MHC class II antigen expression. Transformed and partially dedifferentiated epithelial cell lines obtained by transfection of human thyroid cultures with SV40 DNA sequences have shown the emergence of a DR-positive subpopulation.[12] Furthermore infection *in vitro* of either a rat thyroid or a mouse thyroid-derived epithelial cell line with reovirus types 1 and 3 recently was reported to induce MHC class II (Ia) expression in a variable proportion of the cells.[13,14] While in one of these studies[13] it was not established whether individual Ia-positive cells were actually infected by the virus, in the other[14] induction of Ia expression was linked to the binding of the reovirus (including ultraviolet-inactivated virions) to the cells and was not dependent on virus replication or presence of γ-IFN. Similarly ectopic Ia expression has been induced by noninfective interaction of cultured rat astrocytes with murine hepatitis coronavirus.[15] Here we report that human cytomegalovirus (CMV) infection of primary cultures established from histologically normal thyroid tissue of CMV-seropositive donors results in a widespread induction of ectopic HLA-DR expression on individually uninfected TFCs, associated with the presence of γ-IFN in the supernatants. In addition, CMV directly induces the expression of an abnormal DR or cross-reactive antigen on infected TFCs showing characteristic cytopathic features of late infection, regardless of the immune status of the tissue donor toward CMV.

Materials and Methods

Thyroid Donors and Tissues

Fresh thyroid tissues were obtained from four patients with large nodular goiters in whom partial or subtotal thyroidectomy had been chosen independently as treatment for their condition. The histopathologic diagnoses (Department of Pathology, UCSF) were as follows: involutionary nodule (case 1), multinodular hyperplasia (case 2), and adenomatous goiter (cases 3 and 4). In none of the cases (including case 3, who had previously had a diagnosis of Graves' disease, in complete remission with antithyroid drugs at the time of surgery), were mononuclear cell infiltrates detected in either the nodular or paranodular thyroid tissues. Representative portions from each nodular and paranodular specimen were snap frozen in isopentane at −60°C for cryostat sectioning and the remaining tissue was processed under sterile conditions to obtain dispersed viable cells.

All four tissue donors were typed for the serologically defined HLA-DR allotypic specificities by standard techniques of complement-mediated B-lymphocyte cytotoxicity at the Immunogenetics and Transplantation Laboratory, UCSF. Cytomegalovirus serology status was determined by immunoadherence hemagglutination[16] at Virolab (Berkeley, CA). The presence of circulating thyroid microsomal autoantibodies (TMAb) was assessed by indirect immunofluorescence on autologous viable thyroid monolayers, as previously described.[17]

Cytomegalovirus

Human cytomegalovirus (strain AD 169) was grown and titrated in human fetal lung fibroblast cultures[18] and stored at −80°C at a concentration of 10^7 plaque-forming units (pfu) per milliliter.

Primary Thyroid Cell Monolayers

The method described in previous reports[17,19] was followed. Although an accurate cell count after enzymatic digestion is not feasible, because most viable TFCs are still part of small clusters,[17] parallel cultures from a given cell suspension were approximately equal in terms of cell number. Aliquots of 100 μl of the cell suspension (~5×10^4 cells) were dispensed onto 12-mm round coverslips, placed in 24-well Limbro plates (Flow Laboratories, McLean, VA). After 1 hour of incubation at 37°C, 400 μl of 15% fetal calf serum (FCS) in culture medium was added to each well. In some cases, 10 ml of the cell suspension (~5×10^6 cells) was dispensed and cultured into 75-cm^2 plastic flasks. Cell suspensions from the nodular tissues also were stored frozen in liquid N_2 after slow cooling in 10% dimethylsulfoxide for additional testing.

Cytomegalovirus Infection

Four to eight days after plating, viable thyroid monolayers were washed extensively with culture medium to

remove unattached cells and 200 μl of a freshly made suspension of CMV in culture medium was added to each culture, at a multiplicity of infection of ~1 pfu/cell (ie, 5×10^4 pfu for each coverslip and 5×10^6 pfu for each 75-cm^2 flask). The monolayers were incubated with the CMV inoculum at 37°C for 2 hours with continuous shaking, then washed, and fresh culture medium plus 15% FCS was added to the wells. Control cultures were subjected to the same procedure, but without adding CMV. The cultures were kept in a 5% CO_2 humidified cell incubator until stained by indirect immunofluorescence or radiolabeled with ^{125}I.

Phytohemagglutinin (PHA) Treatment

To induce ectopic DR expression on the TFCs,[5] parallel uninfected cultures were treated with purified phytohemagglutinin (Wellcome Diagnostics, Research Triangle Park, NC) at a final concentration of 10 μg/ml in culture medium plus 15% FCS.

Monoclonal Antibodies

The following monoclonal antibodies (MAb) were used for the detection of various antigens on thyroid follicular and other cell types in the primary thyroid monolayers:

1. Two MAbs, CH16.1[20] and CH167.5,[21] which react with CMV-encoded DNA-binding proteins in infected cells, diluted 1:400
2. Four MAbs directed against nonpolymorphic HLA-DR antigenic determinants: (A) L243,[22] from Becton-Dickinson, Inc., Mountain View, CA, at 5 to 25 μg/ml; (B) 2.06,[23] diluted 1:2; (C) 03-D7,[24] at 30 to 100 μg/ml; and (D) 1800 D2.4,[25] from Mallinckrodt, Inc., St. Louis, MO, at 100 μg/ml
3. MAb Leu 7 (clone HNK-1,[26] from Becton-Dickinson), which recognizes a cell-surface determinant shared by large granular NK lymphocytes, neoplastic/nodular thyroid follicular cells,[27] and other cell types,[28] at 6 to 20 μg/ml
4. Two MAbs directed against cell-surface determinants associated with F_c receptors for IgG on K, NK, and neutrophil leukocytes, from Becton-Dickinson: (A) Leu 11a (clone NKP15,[29] at 50 μg/ml; and (B) Leu 11c (clone B73.1,[30] at 5 to 25 μg/ml

Human Thyroid Autoantibodies

To identify further the TFCs and determine their relative numbers in monolayers from different specimens, CMV-seronegative human sera containing high titers of TMAbs were used in indirect immunofluorescence stainings.[17] In all cases donors of TFCs and serum had the same blood ABO type to avoid binding of natural isoantibodies to ABH antigens re-expressed on the surface of cultured TFCs.[17]

Immunofluorescence (IF) Stainings of Primary Thyroid Cultures

Indirect IF staining of primary thyroid cultures was performed as previously described.[17] After being washed in culture medium, the coverslips were covered with 80 μl of the prediluted (in culture medium plus 15% FCS) MAb or human serum and incubated for 30 minutes at room temperature. This was followed by a second incubation with 80 μl of fluorescein isothiocyanate (FITC)-labeled $F(ab')_2$ fragments of goat anti-mouse IgG, -mouse IgM, or -human IgG, M, A (Cappel, Malvern, PA) diluted 1:40 under similar conditions. All sera and conjugates were ultracentrifuged at 100,000*g* for 10 minutes in an Airfuge (Beckman, Palo Alto, CA) before use, and the monolayers were washed extensively in culture medium after each incubation. The cells then were fixed in 5% acetic acid in ethanol at −20°C for 10 minutes, the coverslips mounted in p-phenylenediamine on a microscope slide, and examined under both phase-contrast and epifluorescence microscopy in a Zeiss photomicroscope (Carl Zeiss, New York, NY). Positive cell-surface stainings were graded + to + + + + according to their brightness, and the proportion of positive cells was estimated after scanning the entire monolayer under X300.

Double-label IF Staining of Cell-surface and Intranuclear Antigens

For this procedure, viable thyroid monolayers first were incubated with anti-DR IgM MAb 1800 D2.4 or human autoimmune serum, followed by FITC-labeled or tetramethylrhodamine isothiocyanate (TRITC)-labeled $F(ab')_2$ fragments of the appropriate goat antiserum, as described above. The cultures then were fixed in chilled acetone for 2 to 4 minutes and exposed to anti-CMV MAbs, CH16.1 or CH167.5, followed by $F(ab')_2$ fragments of goat anti-mouse IgG labeled with the alternative (TRITC or FITC) fluorochrome.[3]

Identification of TFCs in the Primary Cultures

Thyroid follicular cells were identified by their characteristic morphology under phase-contrast microscopy and specific cell-surface reactivity with human TMAbs.[17] Thyroid follicular cells dispersed from thyroid nodules, in

addition to displaying thyroid microsomal antigen (TMAg),[31,32] express a cell-surface antigenic determinant reactive with HNK-1 (Leu 7, Becton-Dickinson) MAb.[27] This determinant, in contrast with TMAg, still can be detected on TFCs cultured for several weeks.[27]

Estimation of the Number of CMV-infected Cells in the Monolayers

This was done by indirect IF staining with a CMV-specific MAb (CH16.1), reactive with the ICP 36 DNA-binding protein[20] and rhodamine-labeled $F(ab')_2$ fragments of goat anti-mouse IgG antibodies on acetone-fixed monolayers, which in most cases also were the substrate for the FITC staining of cell-surface (HLA-DR, HNK-1, or TMAg) antigens, at 2- to 7-day intervals after infection. When the number of CH16.1-positive nuclei within all the X300 microscopic fields along one diameter of the coverslip (12 mm) was 200 or less, the entire coverslip was examined and the total number of CMV-infected cells counted. For those cultures in which the CH16.1-positive nuclei along the diameter initially scanned were more than 200, the total number of infected cells in the monolayer was estimated by multiplying the number counted by 16.3, which is the ratio of the area of the entire coverslip to that of the segment initially scanned. The variation between actual counts and estimates obtained by this procedure, or between estimates obtained by two observers for the same culture, ranged between 7% and 14%.

Prevalence of DR and DR-related Expression on CMV-infected Cells

This was systematically determined by the double-label IF staining of cell-surface DR (with MAb 1800 D2.4 and FITC-labeled goat $F(ab')_2$ anti-mouse IgM), on viable monolayers before fixation, and nuclear CMV-encoded protein (with MAb CH16.1 and TRITC-labeled goat $F(ab')_2$ anti-mouse IgG) on the same culture after acetone fixation. Thyroid follicular cells having a large, usually round or oval, CH16.1-positive intranuclear inclusion were identified under X500 and rhodamine filters; then each cell was examined under FITC filters and scored for cell-surface DR staining. At least 100 CMV-infected TFCs were examined in each culture by two observers and the counts averaged. Fibroblastoid cells, present in small numbers in monolayers cultured from paranodular tissues but virtually absent in those from the nodules, showed signs of earlier and more rapidly spreading CMV infection and their intranuclear inclusions were irregular or kidney shaped.[33] In those cultures in which CMV-infected nonthyroid cells could be identified, their expression of cell-surface DR determinants was assessed in the same way. When anti-DR MAbs of the IgG class were used in IF stainings, cells having single large intranuclear inclusions were identified under phase-contrast microscopy at X500 and then examined for DR expression under FITC filters.

Structural Characterization of CMV-induced Cell-surface HLA-DR Antigens on TFCs

Cell monolayers from the nodular and paranodular thyroid tissues of donor 4 were established in 75-cm^2 plastic flasks at a density of $\sim 5 \times 10^6$ cells/flask, and 4 days later infected with CMV at a multiplicity of infection of ~1 pfu/cell. Seventeen days after infection, CMV-infected and uninfected control monolayers were washed in culture medium and then detached with 0.05% trypsin—0.02% ethylendiamine tetraacetic acid (EDTA) at 37°C. Cell suspensions were centrifuged at 100*g* and pellets resuspended in 20 mmol/l (millimolar) glucose in phosphate-buffered saline (PBS) pH 7.4 at a concentration of 6×10^6 cells/ml. Lactoperoxidase-catalyzed iodination of the viable cells was performed as described by Marchalonis et al.[34] Briefly, lactoperoxidase (Boehringer Mannheim, Indianapolis, IN; 5 mg/ml) was added to the cell suspension (final concentration, 100 μg/ml), followed by the addition of glucose oxidase type V (Sigma Chemical Co., St. Louis, MO; 1400 units/ml) at a final concentration of 50 mU/ml. Na ^{125}I (Amersham, Arlington Heights, IN; 1 mCi/10 μl) then was added to the cells to a final concentration of 0.4 mCi/ml. Cells were kept on ice for 15 minutes with gentle agitation and the reaction then was stopped by adding 5 ml cold PBS containing 0.01 mmol/l sodium metabisulfite and 10 mmol/l sodium azide. After labeling, cells were washed six times in cold PBS and cell membrane proteins were solubilized by mixing the pellet with 0.5% Nonidet P-40 in PBS at 4°C for 15 minutes. The lysates were centrifuged at 10,000*g* at 4°C for 10 minutes and the supernatants collected. ^{125}I labeling of solubilized cell membrane proteins was assessed in a gamma counter (Beckman LS1701). Aliquots of ^{125}I-labeled membrane proteins solubilized from CMV-infected and control cultures, having similar amounts of radioactivity, were precleaned twice by overnight incubations at 4°C with protein A-sepharose beads (CL-4B, Pharmacia, Piscataway, NJ) previously coated with purified rabbit IgG anti-mouse IgG (Dakopatts, Glostrup, Denmark) and mouse control ascites Ig (BRL, Gaithersburg, MD). The preabsorbed supernatants (120 μl) then were incubated sequentially (1 hour at 20°C and overnight at 4°C) with 30 μl each of anti-DR MAb (clones L243 or 1800 D2.4) and rabbit IgG anti-mouse IgG or goat IgG anti-mouse IgG,M (Chemicon, El Segundo, CA). The immunoprecipitates were washed extensively until supernatants did not show

any significant increase in radioactivity above background, then boiled in TRIS buffer 0.0625 mol/l pH 6.8 plus 20% glycerol, 0.004% bromphenol blue and 5% 2-mercaptoethanol, and electrophoresed, together with prestained protein molecular weight standards (BRL), in 11% sodium dodecyl sulfate-polyacrylamide slab gels. Gels were dried and autoradiographed at −70°C for various periods on Kodak X-Omat AR film (Eastman Kodak, Rochester, NY) with an intensifying screen.

Determination of γ-Interferon Levels in Culture Supernatants

A solid-phase enzyme-linked immunosorbent assay (Holland Biotechnology bv, The Netherlands), specifically designed for the quantitation of human γ-IFN in cell culture supernatants (SN), was used. Conditioned SNs of parallel monolayers obtained from the nodular and paranodular thyroid tissue of a CMV-seropositive donor (≠4), and cultured in 75-cm^2 plastic flasks (~5 × 10^6 cells/flask) under each experimental condition (ie, control, CMV-infected, and PHA-treated cultures) were assayed in duplicate after being cleared by centrifugation and concentrated 15-fold (except for SNs from PHA-treated cultures, which were tested unconcentrated) with Centricon-10 microconcentrators (Amicon, Danvers, MA). Supernatants from CMV-infected and PHA-treated cultures were collected when the induction of DR expression on TFCs was manifested fully, as assessed by IF on parallel coverslips (9 days after CMV infection and 3 days after addition of PHA). Control culture SNs were collected 9 days after mock infection. Absorbance readings were made with an automated photocolorimeter (Titertek Multiskan, Flow Laboratories, McLean, VA). Concentrations of γ-IFN in the samples were determined from standard curves obtained by plotting the absorbance of quadruplicate samples of each of four positive controls having known γ-IFN concentrations (ie, 6.25, 12.5, 25, and 50 U/ml) and of a negative control, assayed in parallel.

Results

Cultured TFCs were found to be susceptible to CMV infection, confirming a previous report,[35] with the consequent expression of virally encoded antigens and presence of characteristic cytopathic changes in the infected cells. This infection *in vitro* resulted in the induction of cell-surface expression of DR antigenic determinants on TFCs (Table 1). Two distinct modalities of induction were observed.

Indirect Induction

In CMV-infected cultures from the paranodular (histologically normal) thyroid tissue of the two CMV-seropositive donors, 30% to 50% of the TFCs showed strong induction of DR expression (Figure 1) as early as 3 to 4 days after infection, even though the vast majority of these cells lacked morphologic as well as antigenic evidence of individual infection. This pattern of induction was detected with all four anti-DR MAbs on at least 10 individual cultures from each of these two donors. In contrast, uninfected TFCs in CMV-infected monolayers from either nodular or paranodular thyroid tissue of the two CMV-seronegative donors did not show this widespread induction of DR expression (Table 1). That this induction of DR antigens was seen early on in the course of the slowly progressive CMV infection of the cultures and involved mostly uninfected TFCs strongly suggested the mediation of a diffusible factor. While viral infection stimulates the release of nonimmune (α and β) IFN by many different cell types, neither α-IFN nor β-IFN are able to induce DR expression on human TFCs in culture.[7,8] On the other hand, γ-IFN, which induces HLA class II antigen expression on many cells including TFCs,[7–9] is produced by T lymphocytes in response to specific (ie, antigen) or nonspecific (ie, T-cell mitogens) stimulation.[36] Therefore we measured levels of human γ-IFN in the conditioned culture SNs of parallel 13-day-old monolayers cultured from the nodular and normal paranodular thyroid tissue of a CMV-seropositive (≠4) donor (Figure 2A). While under basal (mock-infected culture) conditions, no γ-IFN could be detected in concentrated (15-fold) SNs from either tissue, significantly increased levels were present in SNs of CMV-infected cultures from normal thyroid tissue. A less pronounced elevation was detected in SNs of CMV-infected nodular thyroid cultures. Furthermore in all cases there was a close correlation between the γ-IFN levels in these supernatants and the proportion of TFCs induced for DR expression in the corresponding monolayers (Figure 2A). In contrast, conditioned SNs of CMV-infected and control thyroid cultures from one of the CMV-seronegative donors (≠3) did not have detectable levels of γ-IFN after concentration. The DR molecule induced on TFCs under these conditions displayed the characteristic subunit profile of classical HLA class II antigens (Figure 2B).

Higher levels of DR induction were observed in uninfected cultures after 3 to 6 days of treatment with PHA, although in this case regardless of the CMV serology of the tissue donors. Furthermore the degree of PHA-induced DR expression on TFCs also was greater in monolayers cultured from normal paranodular tissues than in those from thyroid nodules (Figure 2A). This differential quantitative effect confirms previous results by

Table 1. *Cytomegalovirus-induced Ectopic HLA-DR Expression on Thyroid Cells in Culture*

Thyroid donor	Sex/age	HLA-DR phenotype	CMV serology*	Autologous TMAb† reactivity	Tissue	Cell-surface IF on viable thyroid cultures: Uninfected monolayers 1–2 days after plating — TMAg#	HNK-1	HLA-DR	CMV-infected monolayers 7–14 days PI — HLA-DR on uninfected TFC‡	HLA-DR on infected TFC§
1	M/61	DRw10,w12	1:64	NEG	Nodular	+ + +	+ +/+ + + + 30%	NEG	NEG	25% (874)**
					Paranodular	+ +/+ + +	+ + 1–2%	NEG	+ +/+ + + 40–50%	ND
2	M/36	DR2,4	<1:4	NEG	Nodular	+ +	+ +/+ + + + >80%	NEG	NEG	32% (16,394)
					Paranodular	+/+ +	+ +/+ + + + 20%	NEG	NEG	16% (1585)
3	F/39	DR3	<1:4	+ +	Nodular	+ +	+ +/+ + + + 40%	NEG	NEG	29% (15,844)
					Paranodular¶	+ +	+ + 1–2%	+/+ + <10%	NEG	11% (4742)
4	M/36	DR4	1:16	NEG	Nodular	+ + +	+ + +/+ + + + >90%	+/+ + <5%	+/+ + 5–10%	50% (4035)
					Paranodular	+ +/+ + +	+ +/+ + + + 40%	NEG	+ +/+ + + 30–40%	28% (1824)

* As determined by immune adherence hemagglutination.[16]
† Thyroid microsomal autoantibody as determined by IF on autologous TFC at 1:10 dilution.[17]
‡ As determined by single- and double-label IF with four different anti-DR monoclonal antibodies.
§ As determined by double-label IF with anti-CMV (CH 16-1) and anti-DR (1800 D2.4) monoclonal antibodies.
¶ Graves' disease in complete remission with antithyroid drugs.
# As determined by IF with a blood group-matched, anti–CMV-negative, TMAb-positive human serum at 1:10 dilution.
** Total number of cells in the culture with CMV-positive intranuclear inclusions.
PI, postinfection; ND, not determined.

us [32] and others[6] and further supports the notion that this indirect mechanism of induction, by either specific (CMV in cultures from seropositive donors) or nonspecific (PHA in all cultures) stimuli, would be a function of the numbers of residual immunocompetent cells in the primary monolayers able to respond with γ-IFN release.

Direct Induction

A second type of induction of a DR antigenic determinant on TFCs was observed at a later stage of CMV infection in cultures from all tissues, regardless of the CMV serology of the donors. In infected cultures from the nodular portion of all four thyroid glands, a significant proportion (25% to 50%) of the TFCs showing evidence of advanced CMV infection (large intranuclear inclusions positively stained with CH 16.1 and CH 167.5 MAbs) also displayed a cell-surface coarse granular staining with the anti-DR MAb from clone 1800 D2.4, in double-label IF stainings (Figure 3). The prevalences of CMV-infected TFCs displaying this DR determinant in monolayers cultured from paranodular tissues were somewhat lower (Table 1). MAb 1800 D2.4 (mouse IgM) reacts with a nonpolymorphic epitope that has been shown to be present in human DR molecules by immunoprecipitation, two-dimensional gel electrophoresis, tryptic peptide mapping, and N-terminal amino acid sequence analyses (BD Schwartz, oral personal communication, 1987). In contrast, TFCs with these fully developed cytopathic changes invariably were negative for cell-surface IF staining with the other three anti-DR MAbs, even in cultures derived from the CMV-seropositive donors. Similarly another mouse IgM MAbs (Leu 7, Becton Dickinson), which bound to the cell surface of a large proportion of uninfected TFCs cultured from these nodular tissues (see Khoury et al.[27] and Table 1), failed to react with the TFCs at this advanced stage in their individual CMV infection (data not shown). Cytomegalovirus-infected TFCs with fully developed intranuclear inclusions also were negative for cell-surface expression of Fc receptors for human IgG, as assessed by their lack of staining with MAb Leu 11a and Leu 11c or with autologous serum from one of the CMV-seronegative donors (data not shown). The di-

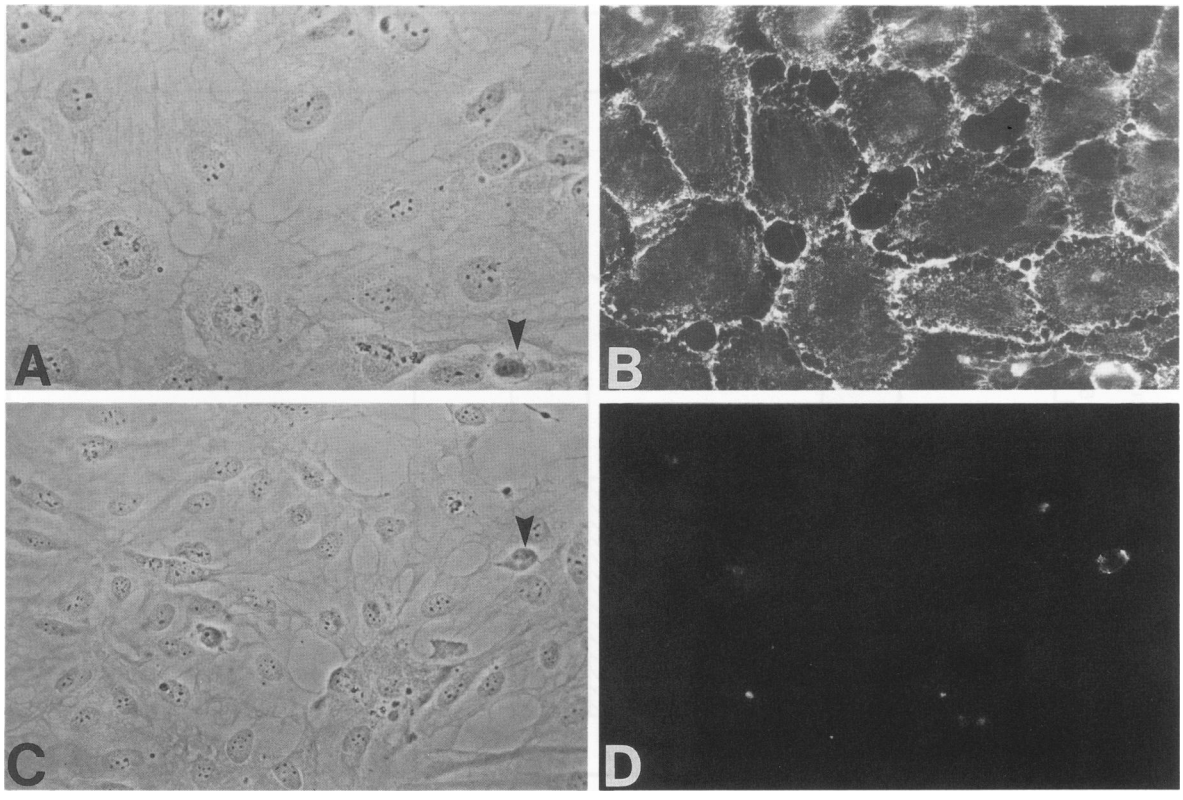

Figure 1. *Widespread induction of cell-surface HLA-DR expression on uninfected TFCs in CMV-infected monolayers cultured from normal thyroid tissue of a CMV-seropositive donor. Indirect IF staining with anti-DR MAb (clone L243), followed by FITC-labeled goat F(ab')$_2$ fragments of anti-mouse IgG, on two parallel 14-day-old monolayers, cultured from the paranodular thyroid tissue of donor 1.* **A:** *CMV-infected culture 7 days after infection. Phase-contrast microscopy showing several spread-out TFCs and one partially spread mononuclear leukocyte (arrowhead); none of the cells in this field have morphologic evidence (ie, intranuclear inclusions) of infection.* **B:** *IF staining on same field as in* **A**; *all TFCs display a dense granular reaction on the cell surface, which is reinforced at the cell borders.* **C:** *Uninfected control monolayer. Phase-contrast microscopy showing many spread-out TFCs and one macrophage (arrowhead).* **D:** *IF staining on same field as in* **C**; *TFCs are completely negative for DR expression and only the macrophage shows cell-surface staining. Original magnifications:* **A** *and* **B**, ×500; **C** *and* **D**, ×300.

rect induction of this DR determinant by CMV on individually infected cells was confined to TFCs, while fibroblastoid cells at a similar stage of infection (large but irregular or kidney-shaped nuclear inclusions[33]) were invariably negative (Figure 4). Thus in cultures from the nodular tissue of donor 4 as many as 42% of the CMV-infected TFCs with large nuclear inclusions showed cell-surface reactivity with MAb 1800 D2.4 seven days after infection (Figure 5B), compared with none of 100 fibroblasts with similarly advanced CMV infection. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of ^{125}I-labeled cell-surface proteins solubilized from the same CMV-infected monolayers used for Figure 2B, but immunoprecipitated with MAb 1800 D2.4 and goat anti-mouse IgG,M serum, revealed three weak bands (not shown). Two of them had the expected mobility of DRα and β subunits, while the third had an apparent molecular weight of about 45 to 50 kd. However, because these CMV-infected cultures were derived from a CMV-seropositive (#4) donor, the contribution to this pattern from (indirectly induced) 'conventional' and (directly induced) 'abnormal' DR antigens, both of which are recognized by MAb 1800 D2.4, could not be determined.

Discussion

The findings described in this report demonstrate that differentiated human thyroid cells can be induced to express MHC class II antigens by CMV infection *in vitro*. Because of the slow progression of the CMV infection with the dose of virus employed and the use of primary thyroid cultures established from either CMV-seropositive or seronegative donors, we were able to obtain evidence that expression of DR antigenic determinants may be induced on both infected and uninfected TFCs in the CMV-infected monolayers. Furthermore the mechanism involved in each of these situations appears to be different and, in the case of uninfected TFCs, the induction is associated with positive CMV serology in the tissue donor and the presence of γ-IFN in the culture supernatants.

Cytomegalovirus infects a large proportion of the hu-

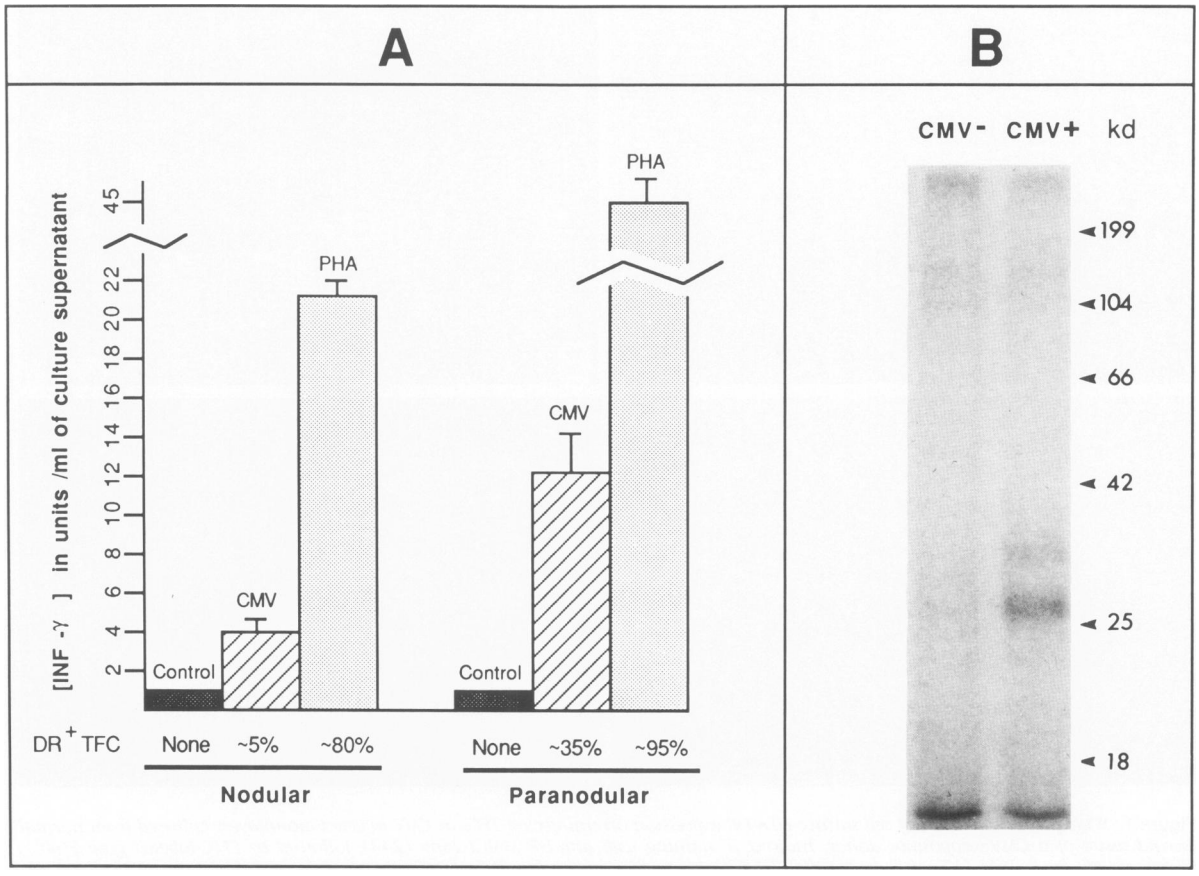

Figure 2. *Characteristics of the HLA-DR induction in CMV-infected thyroid monolayers cultured from a CMV-seropositive donor.* **A:** *Levels (mean ± standard deviation) of γ-interferon in culture supernatants from control, CMV-infected, and PHA-treated monolayers obtained from donor 4 (supernatants from control and CMV-infected cultures were concentrated 15 times). The proportion of DR-positive TFCs in the corresponding cultures is shown below each bar.* **B:** *SDS-polyacrylamide gel electrophoresis of ^{125}I-labeled cell-surface proteins solubilized from two parallel thyroid monolayers, after immunoprecipitation with anti-DR MAb from clone L243.* **Left lane:** *Uninfected culture control.* **Right lane:** *CMV-infected culture; two bands with the apparent mobility of the α (~34 kd) and β (~28 kd) chains of classical DR molecules are present. These bands were not precipitated by the mouse ascites used to preclear the antigen preparation or by irrelevant MAbs used in parallel with MAb L243.*

man population and can cause severe disease in immunocompromised individuals.[37] Histopathologic findings in these patients[38,39] showed that this virus possesses a remarkable tropism *in vivo* for endocrine tissues, particularly adrenal, thyroid, and pancreatic islets. Its ability to induce ectopic HLA class II determinants on infected, as well as neighboring uninfected, thyroid (and possibly other epithelial) cells is likely to have significant implications in 1) normal immune responses to local CMV infections, and 2) the enhancement of alloimmune responses to transplanted grafts. In addition, 3) CMV-induced DR expression on TFCs or other endocrine cells might play a role in the initiation of organ-specific autoimmune responses.

Local Immune Responses to CMV Infection

The normal immune response against foreign (eg, viral) antigenic determinants is initiated and enhanced when specific T inducer/helper lymphocytes recognize those determinants, in association with self MHC class II molecules, on the surface of antigen-presenting cells.[1] Therefore the presence of MHC class II antigens on the surface of a particular cell type might confer a distinct advantage for the containment of infection should those cells become infected by a virus and consequently display virally encoded peptides on their surface. Supporting their putative role as antigen-presenting cells, DR-positive TFCs from Graves' disease tissues were shown to be able to present virally encoded peptides to DR-restricted, antigen-specific T-cell clones *in vitro*.[40] Induction of MHC class II antigens by CMV infection might, therefore, confer on TFCs the capability to participate directly in the generation of the immune response to a viral infection of the thyroid.

In the effector arm of the immune response against CMV-infected cells, the importance of cell-mediated cytotoxicity is well established.[41] To trigger the lysis of a virus-infected cell, the receptor of a sensitized cytotoxic T

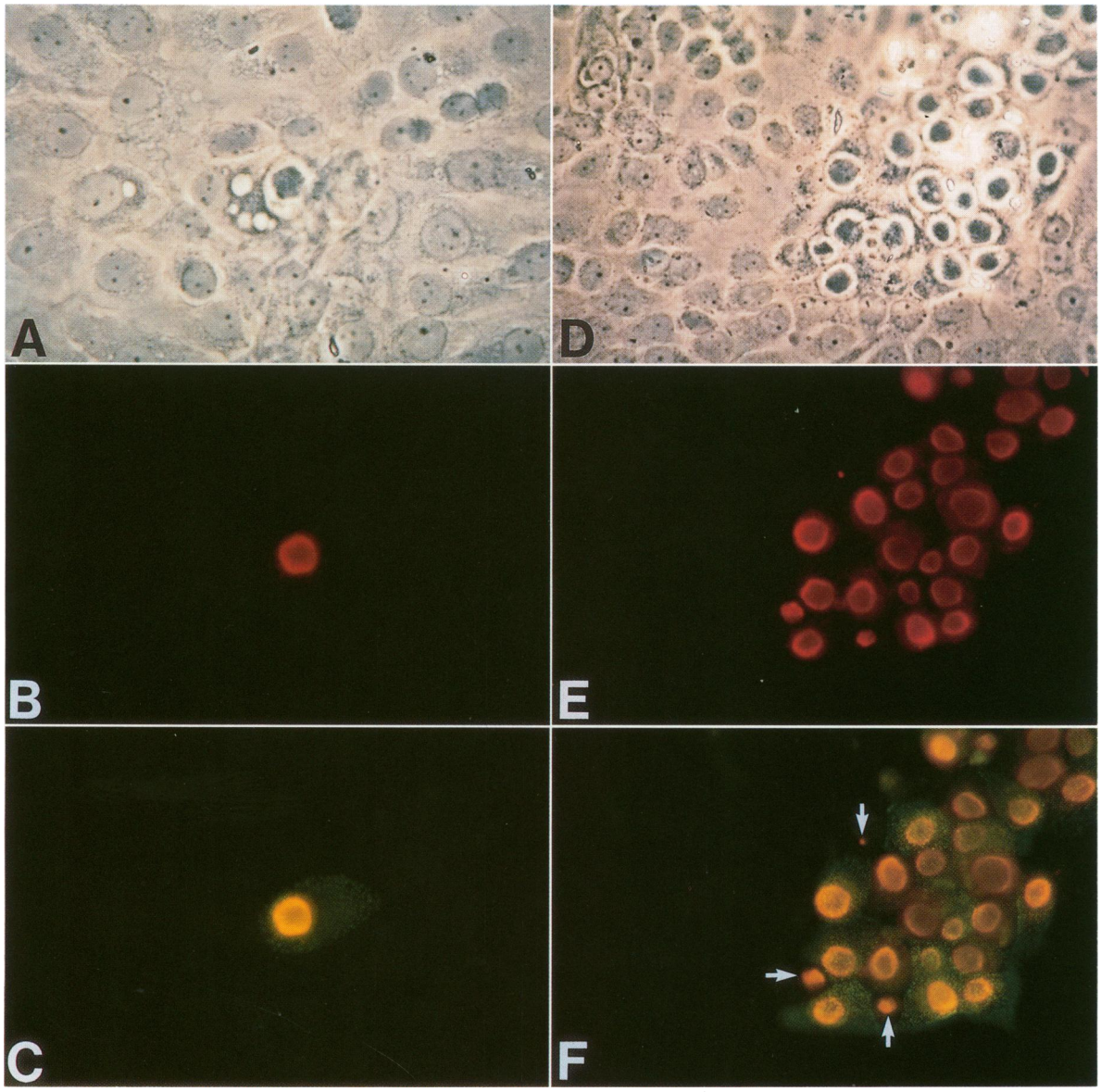

Figure 3. *Direct induction of a cell-surface HLA-DR determinant (1800 D2.4) on CMV-infected TFCs. Double-label IF staining on the same microscopic fields of two (12- and 19-day-old) CMV-infected monolayers cultured from the nodular thyroid tissue of a CMV-seronegative (#2) donor, 7 days* (A–C) *and 14 days* (D–F) *after infection.* A *and* D: *Phase-contrast microscopy showing a homogeneous population of spread-out TFCs; one of the cells in* A *and a cluster of cells in* D *contain large, round, and very dense intranuclear inclusions.* B *and* E: *IF staining given (on permabilized cells, after fixation of the monolayer) by anti-CMV MAb from clone CH16.1, followed by TRITC-labeled goat F(ab')$_2$ fragments anti-mouse IgG. There is a strong positive reaction on the intranuclear inclusions of all the infected TFCs.* C *and* F: *Double-label IF staining given by MAb CH16.1, detected as in* B *and* E, *and (on viable cells, before fixation of the monolayer) by anti-DR MAb from clone 1800 D2.4 followed by FITC-labeled goat F(ab')$_2$ fragments anti-mouse IgM. In addition to the rhodamine staining of the intranuclear inclusions, there is a distinct granular FITC reaction on the surface of the CMV-infected TFC in* C *and on many of the infected TFCs in* F. *Note that the cell-surface FITC staining is coarser and sparser than that due to indirect DR induction on uninfected TFCs in infected cultures from CMV-seropositive donors (Figures 1B and 4C). The strong TRITC staining of the viral inclusions obscures the cell-surface–bound FITC granules above the nuclear areas. Those cells at an earlier stage in the infection and having smaller inclusions are negative for this direct induction (arrows). All uninfected TFCs are also negative. Original magnification, ×500.*

lymphocyte usually recognizes a complex between the specific viral antigen and the autologous MHC class I molecule.[42,43] In common with certain other viral pathogens in humans, CMV has been shown to bind to MHC class I molecules on the host-cell surface as the initial step in infection.[44] In infected cells, binding of CMV constituents to MHC class I antigens, therefore, may result in the functional blocking of the latter with the consequent impairment of T-cell–mediated lysis through this pathway. Indeed we obtained evidence of absence or blocking of the cell-surface expression of both the MHC class I heavy chain and β_2 microglobulin on CMV-infected TFCs with fully developed nuclear inclusions (EL Khoury, unpublished results). In this context, a concomitant induction of ectopic MHC class II antigens would be particularly relevant because it has been shown that T-

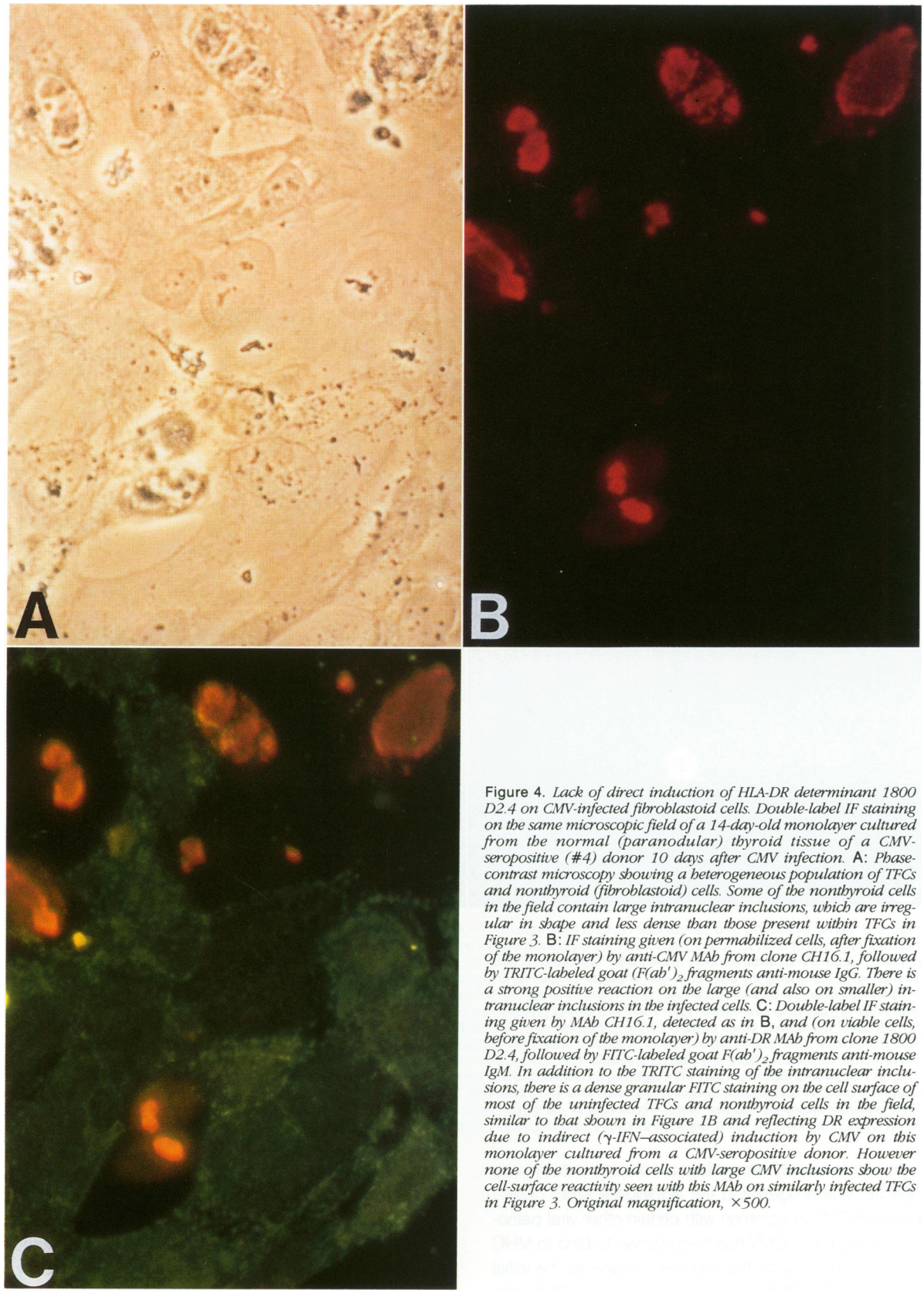

Figure 4. *Lack of direct induction of HLA-DR determinant 1800 D2.4 on CMV-infected fibroblastoid cells. Double-label IF staining on the same microscopic field of a 14-day-old monolayer cultured from the normal (paranodular) thyroid tissue of a CMV-seropositive (#4) donor 10 days after CMV infection.* **A**: *Phase-contrast microscopy showing a heterogeneous population of TFCs and nonthyroid (fibroblastoid) cells. Some of the nonthyroid cells in the field contain large intranuclear inclusions, which are irregular in shape and less dense than those present within TFCs in Figure 3.* **B**: *IF staining given (on permabilized cells, after fixation of the monolayer) by anti-CMV MAb from clone CH16.1, followed by TRITC-labeled goat $(F(ab')_2$ fragments anti-mouse IgG. There is a strong positive reaction on the large (and also on smaller) intranuclear inclusions in the infected cells.* **C**: *Double-label IF staining given by MAb CH16.1, detected as in* **B**, *and (on viable cells, before fixation of the monolayer) by anti-DR MAb from clone 1800 D2.4, followed by FITC-labeled goat $F(ab')_2$ fragments anti-mouse IgM. In addition to the TRITC staining of the intranuclear inclusions, there is a dense granular FITC staining on the cell surface of most of the uninfected TFCs and nonthyroid cells in the field, similar to that shown in Figure 1B and reflecting DR expression due to indirect (γ-IFN–associated) induction by CMV on this monolayer cultured from a CMV-seropositive donor. However none of the nonthyroid cells with large CMV inclusions show the cell-surface reactivity seen with this MAb on similarly infected TFCs in Figure 3. Original magnification, ×500.*

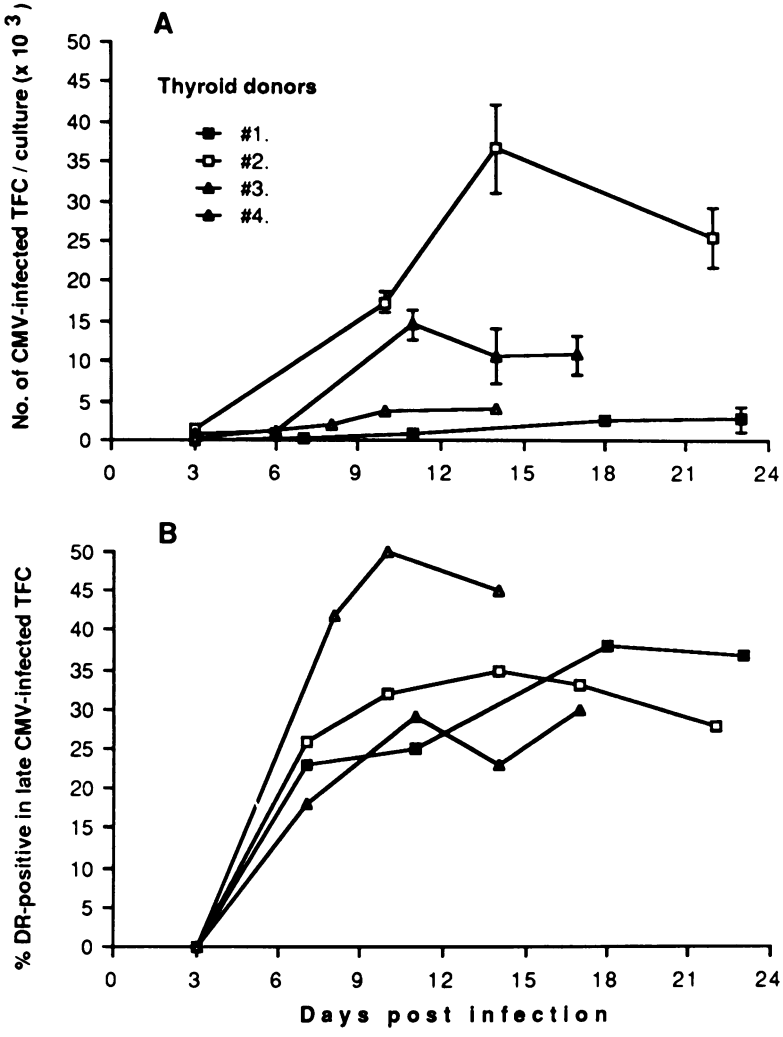

Figure 5. *Time course, in monolayers cultured from nodular thyroid tissues of the four donors and infected with CMV* in vitro, *of:* **A**: *The progression of the CMV infection, as determined by the numbers of CMV-positive TFCs per culture (~5 × 10⁴ cells). Each data point represents the mean ± standard deviation (SD) of the number of TFCs having MAb CH16.1-positive IF staining on their nuclear inclusions, estimated in two or three parallel monolayers as described in* Materials and Methods. *Data points without a SD bar had SD values too small to be depicted with the scale used. Note that monolayers obtained from the CMV-seronegative (#2 and #3) donors consistently showed higher numbers of infected cells than those from the CMV-seropositive donors.* **B**: *The prevalence of cell-surface expression of the HLA-DR determinant recognized by MAb 1800 D2.4 among those individual TFCs showing evidence of advanced CMV infection (ie, fully developed intranuclear inclusions positively stained with MAb CH16.1), as determined by double-label IF stainings. Each data point represents the average of two values determined by different observers, screening at least 100 CMV-infected TFCs each, in the same monolayer. Note that, in contrast to the indirect HLA-DR induction, which was seen only in monolayers cultured from CMV-seropositive donors, this direct induction was present on infected TFCs of cultures from either CMV-seropositive or -seronegative donors with comparable prevalences.*

lymphocyte–mediated lysis of some CMV-infected human cells is DR (and not class I) restricted.[45] Human leukocyte antigen class II restriction also was described for measles[46] and mumps[47] virus-specific T cytotoxic lymphocytes.

Our findings provide evidence for an unexpectedly high frequency of CMV-sensitized T lymphocytes in primary cultures from normal nonlymphoid tissue of unselected CMV-seropositive individuals. The frequency of CMV-specific T cytotoxic precursor cells recently was estimated to be 1:5000 to 1:20,000 of peripheral blood T lymphocytes.[48] On the other hand, the frequency of residual T lymphocytes in primary cultures established from normal human thyroid tissues, under similar conditions to those used in this study, was determined to be approximately 1.6% of the total number of cells in the monolayers.[6] Because each of our cultures had about 5 × 10^4 cells, the frequency of primed T cells in those from CMV-seropositive donors, able to respond to CMV *in vitro* with release of γ-IFN resulting in widespread DR induction on uninfected TFCs, appears to be much higher (≥1:800) than what would have been expected. An additional source of γ-IFN in the thyroid cultures might result from the recruitment of natural killer (NK) cells, which are known to produce γ-IFN when stimulated by interleukin-2 released by activated T lymphocytes.[36] Thus the antigen-specific activation of a small number of CMV-sensitized T cells in monolayers cultured from CMV-seropositive donors could trigger the release by antigen-nonspecific NK cells of sufficient γ-IFN to mediate DR induction on neighboring TFCs. Production of γ-IFN is a highly characteristic component of the immune response *in vitro* to murine CMV by lymphocytes cultured from previously infected animals.[49] The γ-IFN levels associated with the indirect DR induction in CMV-infected monolayers were modest and their reproducible quantitation required concentrating the SNs. This may reflect the fact that the SNs were assayed when DR expression was already fully manifested (9 days after infection) and, therefore, after the peak in local γ-IFN[10] presumably responsible for the induction. Although 10 to 100 U/ml is the usual range of recombinant γ-IFN used for inducing DR antigens on

TFCs *in vitro*, a concentration as low as 1 U/ml has been shown to induce this expression on up to 30% of TFCs in the treated monolayers.[7] Because we did not determine the effect of anti–γ-IFN antibodies on this indirect induction, a possible mediation by other soluble factor(s) cannot be completely excluded. In any case, the release of any alternative inducing factor would also have been dependent on immune recognition of CMV in the infected cultures. Widespread foci of MHC class II positive but uninfected cells have been observed also in the brains of rats infected with measles virus *in vivo*,[50] possibly reflecting a similar mechanism of induction. Furthermore a putative *in vitro* immune response also might explain the consistently lower numbers of infected cells seen in cultures from seropositive donors after infection with equal doses of CMV (Figure 5A).

The characterization of the cell-surface molecule bearing the HLA-DR determinant detected by MAb 1800 D2.4 on CMV-infected TFCs with fully developed cytopathic changes, and the elucidation of the mechanism responsible for its direct induction, requires further study. In contrast to the recently described Ia induction in a murine thyroid-derived epithelial cell line by reovirus,[14] the direct induction of this DR-related antigen by CMV did require viral replication because it was present only on TFCs displaying fully developed cytopathic changes. Major histocompatibility complex class II antigens might be induced as a consequence of transcriptional activation of their encoding genes in the infected TFCs. Cytomegalovirus infection of human cells *in vitro* is known to stimulate the synthesis of certain cellular enzymes due to a selective increase in mRNA transcripts from their corresponding genes.[51] Induction of a 'defective' DR molecule (ie, recognized by some but not all MAbs directed against its nonpolymorphic determinants) might be the consequence of either an uncoordinated transcriptional activation of MHC class II genes or the expression of a host cell-encoded cross-reactive protein in infected TFCs. Alternatively the lack of reactivity with most anti-DR MAbs might be due to post-translational blocking of DR epitopes by CMV-encoded products at this late stage in infection. The failure to reproduce the cell-surface reactivity of MAb 1800 D2.4 on CMV-infected TFCs with MAb Leu 11a or Leu 11c, or with autologous serum from a CMV-seronegative donor, would indicate that this epitope is not part of Fc receptors for human IgG induced by the CMV infection.[52] On the other hand, it was reported that a protein encoded by the CMV immediate early (IE) 2 gene shares a common pentapeptide sequence with the β chain of DR molecules and that rabbits immunized with this homologous sequence generated antibodies that recognize a common epitope in CMV-infected human fibroblasts and DR β chains from uninfected lymphoblastoid cells.[53] This immunologic cross-reactivity might be construed to be the mechanism responsible for the appearance of the nonpolymorphic DR determinant detected by MAb 1800 D2.4 on a proportion of CMV-infected TFCs. However two facts argue against this possibility. First CMV IE genes are expressed immediately after infection and their products are localized to the nucleus, while the DR determinant detected by MAb 1800 D2.4 is seen on the surface of cells only at a late stage of infection. Second this direct DR induction on infected cells is confined to TFCs and CMV-infected fibroblasts present in the same primary cultures remain negative even at advanced stages of infection. Furthermore transfection of thyroid monolayers cultured from donor 4 with plasmids carrying the CMV IE2A gene, either alone or together with the IE1 gene, failed to induce cell-surface expression of the determinant recognized by MAb 1800 D2.4 on TFCs displaying the products of these transfected CMV genes (Khoury EL, Mocarski ES, unpublished results).

Enhancement of Immune Responses to Allografts

Induction of HLA class II expression by CMV on other differentiated epithelial cells, for example those in kidney tubules, may play a role in allograft rejection. Cytomegalovirus infection is a serious complication in kidney recipients, frequently leading to graft rejection.[54] During rejection episodes there is an enhanced HLA class II antigen expression in the parenchymal (vascular endothelial and tubular) cells of the graft.[55] Furthermore CMV infection of the graft was shown to be invariably associated with up-regulation of DR expression and it has been proposed that induction of HLA class II expression on parenchymal cells, presumably mediated by release of γ-IFN as a consequence of CMV infection, is the reason for increased graft rejection associated with CMV.[56] Fujinami et al[53] suggested the alternative possibility that a CMV/DR cross-reactive epitope is expressed on CMV-infected parenchymal cells in the graft. Our findings on CMV-infected endocrine cell cultures are consistent with the operation of either of these mechanisms, although the putative cross-reactive determinant detected by MAb 1800 D2.4 appears to be host cell and not CMV encoded.

Generation of Organ-specific Autoimmune Responses

A role for viral infection in the etiology of endocrine autoimmune disorders has long been postulated. This notion has drawn support from the description of virally induced endocrinopathies with autoimmune markers in ex-

perimental models.[57] One of the various mechanisms whereby viral agents would trigger autoimmunity might operate through the induction of ectopic MHC class II antigens. It has been proposed[11] that this induction would enable the endocrine cells to behave as autoantigen-presenting cells and efficiently present, to potentially autoreactive MHC class II-restricted T helper lymphocytes, self constituents that would otherwise remain silent.

Several humoral markers of autoimmunity have been reported to appear as a consequence of human[58] and experimental[59] CMV infection *in vivo*. On the other hand, we[32] and others[9] obtained clear evidence that MHC class II expression detectable on TFCs of thyroid glands affected by ongoing autoimmune phenomena is not a primary or intrinsic abnormality of the TFCs but rather a consequence of their response to the lymphoid infiltration and local release of lymphokines. This would rule out a 'direct-type' DR induction on TFCs, resulting from their latent or persistent infection with CMV, as an initial step in the development of thyroid autoimmunity. Nevertheless the possibility still remains of an initial 'indirect-type' (through γ-IFN) induction by a local viral infection, subsequently maintained by the autoreactive lymphoid infiltrates. The reported high prevalence of persistent CMV infection in newly diagnosed cases of type I diabetes mellitus with islet cell autoantibodies[60] might reflect that possibility.

Acknowledgments

The authors thank Dr. Orlo H. Clark for making available the surgical specimens, Dr. Evelyn T. Lennette for performing the CMV serology, Dr. Marvin Garovoy for HLA typing, Dr. Hugh McDevitt for providing monoclonal antibody 2.06, Dr. Pierre De Meyts for his gift of monoclonal antibody 03-D7, Dr. Phil Vorwald for providing monoclonal antibody Leu IIa in concentrated form, Dr. Jeffrey R. Felton and Dr. Edward S. Mocarski for valuable discussions, Jennifer W. Berline for technical assistance, and Nancy Frost for preparing the manuscript.

References

1. Unanue ER, Allen PM: The basis for the immunoregulatory role of macrophages and other accessory cells. Science 1987, 236:551–557
2. Klareskog L, Forsum U: Tissue distribution of class II transplantation antigens: Presence in normal cells. *In* Solheim BG, Møller E, Ferrone S, eds. HLA Class II Antigens: A Comprehensive Review of Structure and Function. Berlin, Springer-Verlag, 1986, pp 339–355
3. Khoury EL, Greenspan JS, Greenspan FS: Adrenocortical cells of the zona reticularis normally express HLA-DR antigenic determinants. Am J Pathol 1987, 127:580–591
4. Hanafusa T, Pujol-Borrell R, Chiovato L, Russell RCG, Doniach D, Bottazzo GF: Aberrant expression of HLA-DR antigen on thyrocytes in Graves' disease: Relevance for autoimmunity. Lancet 1983, ii:1111–1115
5. Pujol-Borrell R, Hanafusa T, Chiovato L, Bottazzo GF: Lectin-induced expression of DR antigen on human cultured follicular thyroid cells. Nature 1983, 304:71–73
6. Piccinini LA, Mackenzie WA, Platzer M, Davies TF: Lymphokine regulation of HLA-DR gene expression in human thyroid cell monolayers. J Clin Endocrinol Metab 1987, 64:543–548
7. Todd I, Pujol-Borrell R, Hammond LJ, Bottazzo GF, Feldmann M: Interferon-γ induces HLA-DR expression by thyroid epithelium. Clin Exp Immunol 1985, 61:265–273
8. Weetman AP, Volkman DJ, Burman KD, Gerrard TL, Fauci AS: The *in vitro* regulation of human thyrocyte HLA-DR antigen expression. J Clin Endocrinol Metab 1985, 61:817–824
9. Iwatani Y, Gerstein HC, Iitaka M, Row VV, Volpe R: Thyrocyte HLA-DR expression and interferon-γ production in autoimmune thyroid disease. J Clin Endocrinol Metab 1986, 63:695–708
10. Collins T, Pober JS, Strominger JL: Physiologic regulation of class II major histocompatibility complex gene expression. *In* Solheim BG, Møller E, Ferrone S, eds. HLA Class II Antigens: A Comprehensive Review of Structure and Function. Berlin, Springer Verlag, 1986, pp 14–31
11. Bottazzo GF, Pujol-Borrell R, Hanafusa T, Feldmann M: Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. Lancet 1983, ii:1115–1119
12. Todd I, Pujol-Borrell R, Belfiore A, Bottazzo GF: Thyrocyte HLA class II expression and regulation in relation to thyroid autoimmunity. Acta Endocrinol (Copenh) Suppl 1987, 281:27–34
13. Neufeld DS, Platzer M, Davies TF: Reovirus induction of MHC class II antigen in rat thyroid cells. Endocrinology 1989, 124:543–545
14. Gaulton GN, Stein ME, Safko B, Stadecker MJ: Direct induction of Ia antigen on murine thyroid-derived epithelial cells by reovirus. J Immunol 1989, 142:3821–3825
15. Massa PT, Dörries R, ter Meulen V: Viral particules induce Ia antigen expression on astrocytes. Nature 1986, 320:543–546
16. Dienstag JL, Cline WL, Purcell RH: Detection of cytomegalovirus antibody by immune adherence hemagglutination. Proc Soc Exp Biol Med 1976, 153:543–548
17. Khoury EL, Hammond L, Bottazzo GF, Doniach D: Presence of the organ-specific "microsomal" autoantigen on the surface of human thyroid cells in culture: Its involvement in complement-mediated cytotoxicity. Clin Exp Immunol 1981, 45:316–328
18. Pereira L, Hoffman M, Cremer N: Electrophoretic analysis of polypeptides immune precipitated from cytomegalovirus-infected cell extracts by human sera. Infect Immun 1982, 36:933–942
19. Khoury EL, Bottazzo GF, Roitt IM: The thyroid microsomal antibody revisited. Its paradoxical binding *in vivo* to the api-

cal surface of the follicular epithelium. J Exp Med 1984, 159:577–591

20. Mocarski ES, Pereira L, Michael N: Precise localization of genes on large animal virus genomes: Use of λgt11 and monoclonal antibodies to map the gene for a cytomegalovirus protein family. Proc Natl Acad Sci USA 1985, 82:1266–1270
21. Kemble GW, McCormick L, Pereira L, Mocarski ES: A cytomegalovirus protein with properties of herpes simplex virus ICP8: Partial purification of the polypeptide and map position of the gene. J Virol 1987, 61:3143–3151
22. Lampson LA, Levy R: Two populations of Ia-like molecules on a human B cell line. J Immunol 1980, 125:293–299
23. Charron DJ, McDevitt HO: Analysis of *HLA-D* region-associated molecules with monoclonal antibody. Proc Natl Acad Sci USA 1979, 76:6567–6571
24. DeMeyts P, Hallez S, Gu JL, Merchez M, Economidis I, Rousseau GG, van Snick J, Spinel C, Squifflet JP, Ravoet AM, de Bruyere M, Willard K, Vessiere F, Lemoine C, Boon T, Toyoda H, Chen F, Shively J: Aberrant expression of HLA-DR determinants on human thyroid cells treated with phytohemagglutinin and gamma-interferon and on human melanoma cells. *In* Schwartz R, Rose NR, eds. Autoimmunity: Experimental and Clinical Aspects. New York, New York Academy of Sciences, 1986, pp 359–360
25. Zacheis M, Giacoletto K, Schwartz BD: Analysis of normal human tonsil class II antigen glycosylation by lectin affinity chromatography. J Biol Chem 1986, 261:17004–17010
26. Abo T, Balch CM: A differentiation antigen of human NK cells identified by a monoclonal antibody (HNK-1). J Immunol 1981, 127:1024–1029
27. Khoury EL, Greenspan FS, Greenspan JS: Shared cell-surface antigenic determinant on neoplastic thyroid follicular cells and natural killer lymphocytes. 68th Ann Meet Endocr Soc, Anaheim, CA, 1986(Abstr)
28. Lipinski M, Braham K, Caillaud JM, Carlu C, Tursz T: HNK-1 antibody detects an antigen expressed on neuroectodermal cells. J Exp Med 1983, 158:1775–1780
29. Phillips JH, Babcock GF: NK-15: A monoclonal antibody reactive against purified human natural killer cells and granulocytes. Immunol Letters 1983, 6:143–149
30. Perussia B, Starr S, Abraham S, Fanning V, Trinchieri G: Human natural killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor functions. 1. Characterization of the lymphocyte subset reactive with B73.1. J Immunol 1983, 130:2133–2141
31. Doniach I, Perrin J: Distribution of microsomal antigen in various types of thyroid tumor. Clin Exp Immunol 1973, 14:77–90
32. Khoury EL, Greenspan JS, Greenspan FS: Ectopic expression of HLA class II antigens on thyroid follicular cells: Induction and transfer *in vitro* by autologous mononuclear leukocytes. J Clin Endocrinol Metab 1988, 67:992–1004
33. Albrecht TB, Cavallo T, Cole BS, Graves K: Cytomegalovirus: Development and progression of cytopathic effects in human cell culture. Lab Invest 1980, 42:1–7
34. Marchalonis JJ, Cone RE, Santer V: Enzymatic iodination. A probe for accessible surface proteins of normal and neoplastic lymphocytes. Biochem J 1971, 124:921–927
35. Knowles WA: *In vitro* cultivation of human cytomegalovirus in thyroid epithelial cells. Arch Virol 1976, 50:119–124
36. Trinchieri G, Perussia B: Immune interferon: A pleiotropic lymphokine with multiple effects. Immunol Today 1985, 6:131–136
37. Ho M: Characteristics of cytomegalovirus. *In* Ho M, ed. Cytomegalovirus: Biology and Infection. New York, Plenum Press, 1982, pp 9–32
38. Niedt GW, Schinella RA: Acquired immunodeficiency syndrome. Clinicopathologic study of 56 autopsies. Arch Pathol Lab Med 1985, 109:727–734
39. Welch K, Finkbeiner W, Alpers CE, Blumenfeld W, Davis RL, Smuckler EA, Beckstead JH: Autopsy findings in the acquired immune deficiency syndrome. JAMA 1984, 252:1152–1159
40. Londei M, Lamb JR, Bottazzo GF, Feldmann M: Epithelial cells expressing aberrant MHC class II determinants can present antigen to cloned human T cells. Nature 1984, 312:639–641
41. Quinnan GV Jr, Rook AH: The importance of cytotoxic cellular immunity in the protection from cytomegalovirus infection. *In* Plotkin SA, Michelson S, Pagano JS, eds. CMV: Pathogenesis and Prevention of Human Infection. March of Dimes Birth Defects, Vol. 20, No. 1. New York, AR Liss, 1984, pp 245–261
42. McMichael AJ, Ting A: HLA restriction of cell-mediated lysis of influenza virus-infected human cells. Nature 1977, 270:524–526
43. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC: The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature 1987, 329:512–518
44. Grundy JE, McKeating JA, Ward PD, Sanderson AR, Griffiths PD: β_2 microglobulin enhances the infectivity of cytomegalovirus and when bound to the virus enables class I HLA molecules to be used as a virus receptor. J Gen Virol 1987, 68:793–803
45. Lindsley MD, Torpey DJ III, Rinaldo CR Jr: HLA-DR-restricted cytotoxicity of cytomegalovirus-infected monocytes mediated by Leu-3-positive T cells. J Immunol 1986, 136:3045–3051
46. Jacobson S, Sekaly RP, Jacobson CL, McFarland HF, Long EO: HLA class II-restricted presentation of cytoplasmic measles virus antigens to cytotoxic T cells. J Virol 1989, 63:1756–1762
47. Goodman AD, Jacobson S, McFarland HF: Mumps virus-specific cytotoxic T lymphocyte responses can be mediated by class II MHC-restricted $CD4^+$ cells. J Neuroimmunol 1987, 16:65(Abstr)
48. Borysiewicz LK, Graham S, Hickling JK, Mason PD, Sissons JGP: Human cytomegalovirus-specific cytotoxic T cells: Their precursor frequency and stage specificity. Eur J Immunol 1988, 18:269–275
49. Rytel MW, Hooks JJ: Induction of immune interferon by murine cytomegalovirus. Proc Soc Exp Biol Med 1977, 155: 611–614

50. Olsson T, Maehlen J, Love A, Klareskog L, Norrby E, Kristensson K: Induction of class I and class II transplantation antigens in rat brain during fatal and non-fatal measles virus infection. J Neuroimmunol 1987, 16:215–224
51. Colberg-Poley AM, Santomenna LD: Selective induction of chromosomal gene expression by human cytomegalovirus. Virology 1988, 166:217–228
52. Sukuma S, Furukawa T, Plotkin SA: The characterization of IgG receptor induced by human cytomegalovirus. Proc Soc Exp Biol Med 1977, 155:168–172
53. Fujinami RS, Nelson JA, Walker L, Oldstone MB: Sequence homology and immunologic cross-reactivity of human cytomegalovirus with HLA-DR beta chain: A means for graft rejection and immunosuppression. J Virol 1988, 62:100–105
54. Peterson PK, Balfour HH, Marker SC, Fryd DS, Howard RJ, Simmon RL: Cytomegalovirus disease in renal allograft recipients: A prospective study of the clinical features, risk factors and impact on renal transplantation. Medicine 1980, 59:283–300
55. Hayry P, von Willebrand E: The influence of the pattern of inflammation and administration of steroids on class II MHC antigen expression in renal transplants. Transplantation 1986, 42:358–363
56. von Willebrand E, Pettersson E, Ahonen J, Hayry P: CMV infection, class II antigen expression, and human kidney allograft rejection. Transplantation 1986, 42:364–367
57. Srinivasappa J, Garzelli C, Onodera T, Ray UR, Notkins AL: Virus-induced thyroiditis. Endocrinology 1988, 122:563–566
58. Andersen P, Andersen HK: Smooth-muscle antibodies and other tissue antibodies in cytomegalovirus infection. Clin Exp Immunol 1975, 22:22–29
59. Bartholomaeus WN, O'Donoghue H, Foti D, Lawson CM, Shellam GR, Reed WD: Multiple autoantibodies following cytomegalovirus infection: Virus distribution and specificity of autoantibodies. Immunology 1988, 64:397–405
60. Pak CY, McArthur RG, Hyone-Myong E, Yoon JW: Association of cytomegalovirus infection with autoimmune type 1 diabetes. Lancet 1988, ii:1–4