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Renal allograft rejection: investigation of alloantigen presentation by cultured human renal epithelial cells

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SUMMARY

Defined lines of primary human renal epithelial cells were established and their expression of class II major histocompatibility (MHC) antigens was up-regulated by culture with interferon-gamma (IFN-γ). The ability of these cells to stimulate the proliferation of allogeneic peripheral blood mononuclear cells (PBMC) was compared with that of endothelial cells and splenic mononuclear cells. It was found that both endothelial and splenic cells stimulated lympho-proliferation but that cultured renal epithelial cells were non-stimulatory. The failure of proliferation by allogeneic lymphocytes in culture with epithelial cells was not overcome by treatment with interleukin-1 (IL-1) or indomethacin. However, addition of IL-2 to mixed cultures of allogeneic PBMC and renal epithelial cells stimulated lympho-proliferation and allowed the generation of lymphoid cell lines which mediated non-specific lysis of renal epithelial cell lines. Stimulation of PBMC by mixed lymphocyte culture yielded an allospecific T-cell line which was added either to renal epithelial cells from the same donor as the stimulator cells used in the priming reaction or from a third-party donor; lympho-proliferation was observed in the specific secondary reaction but not in the non-specific reaction. These findings indicate that class II MHC antigen-expressing epithelial cells within a renal allograft may not initially stimulate the proliferation of resting allospecific recipient lymphoctytes. However, within a rejecting graft it is likely that high local concentrations of IL-2 are present and that many of the infiltrating allospecific lymphocytes will be primed by previous contact with donor antigen-presenting cells, such as vascular endothelial cells or dendritic cells. Therefore, expression of class II MHC antigens by epithelial cells within the microenvironment of a renal allograft may render such cells immunogenic and able to play a direct role in the lymphocyte-mediated intragraft rejection process.

INTRODUCTION

It is generally accepted that organ allograft rejection occurs as the result of an interaction between allospecific T lymphocytes derived from the recipient and a complex formed between donor major histocompatibility antigens (MHC) and peptide fragments which may also have a donor origin.[1]

In view of the pivotal role ascribed to class II MHC-restricted CD4-positive 'helper' T lymphocytes in the initiation of immune responses, much work has centred on analysis of the intra-allograft disposition of cells expressing class II MHC antigens during periods of acute graft rejection.[2,3] It is clear that such cells fall into two categories. The first category consists of the antigen-presenting cells (APC) which include bone marrow-derived cells such as macrophages, dendritic cells and B lymphocytes, which constitutively express class II MHC antigens, and vascular endothelial cells, which may be induced to express class II MHC antigens. All of these cell types are able to stimulate the proliferation of antigen-specific lymphocytes.[4–6] The second category consists of parenchymal cells such as epithelial cells and fibroblasts which are not normally considered to be antigen-presenting cells but which can express high levels of class II MHC antigens when stimulated *in vitro* by exposure to cytokines such as interferon-gamma (IFN-γ).[7–9] It is possible that such class II MHC antigen-expressing cells may present a wide range of alloantigenic stimuli, including tissue-restricted and minor histocompatibility antigens, to allograft infiltrating lymphocytes and thereby amplify and direct the rejection of transplanted organs. For example, the appearance of class II MHC antigens on bronchial epithelial cells has been implicated in the development of obliterative bronchiolitis following experimental lung transplantation[10] and the expression of class II antigens by tubular epithelial cells coincides

Abbreviations: APC, antigen-presenting cells; HUVEC, human umbilical vein endothelial cells; MLKC, mixed lymphocyte kidney culture.

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with necrosis of the tubular epithelium during acute renal allograft rejection.

It has been reported that IFN-γ-treated human or canine renal cells *in vitro* can stimulate the proliferation of allogeneic lymphocytes in the so-called mixed lymphocyte kidney culture (MLKC).[11-13] However, conflicting results have also appeared in which little lymphocyte proliferation is observed in this sytem.[14] In this report the ability of defined populations of long-term cultured human renal epithelial cells to stimulate the proliferation of allogeneic lymphocytes after treatment with a variety of cytokines is investigated and the results are compared with those of known antigen-presenting cells such as splenic cells and human umbilical vein endothelial cells (HUVEC).

MATERIALS AND METHODS

Culture of renal epithelial cells

Kidney specimens were removed either from renal allograft recipients experiencing irreversible graft rejection or from patients with renal cell carcinoma. Cortical tissue was dissected into heparinized RPMI-1640 culture medium (Northumbria Biologicals, Cramlington, Northumberland, U.K.) and stored at 4° before processing.

Tubular epithelial cells were derived using the technique described by Kirby *et al.*[15] Briefly, the cortical tissue was cut into 2 mm^3 blocks which were pressed through a 180 μm stainless steel mesh (Endecotts Ltd, Lond, U.K.). The tissue fragments eluted from this mesh by a continuous stream of RPMI-1640 were separted into glomerular and tubular fractions by serial retention on 106 μm and 45 μm meshes, respectively. The tubular fraction was cultured in horizontal 25 cm^2 flasks (Falcon, Becton-Dickinson, Cowley, Oxon, U.K.) in 'complete' RPMI-1640 medium containing 10% (v/v) heat-inactivated foetal bovine serum, HEPES buffer (pH 7·3; 10×10^{-3} M), ampicillin (100 μg/ml) and streptomycin (100 μg/ml), which was additionally supplemented with insulin-transferrin-sodium selenite (Sigma Chemicals, Poole, Dorset, U.K.), tri-iodothyronine (3×10^{-8} M; Sigma) and hydrocortisone (5×10^{-8} M; Sigma). Confluent cultures were split in the ratio 1:3 as necessary using trypsin-EDTA (Northumbria Biologicals) to release the adherent cells. On the second pass the cells were cultured in D-valine-containing medium in order to prevent proliferation of non-epithelial cells.[16]

The kidney cells used in this series of experiments were all collected from passes 3 to 8. Samples of the cells cultured from each kidney specimen were propagated on glass slides and were characterized by morphological investigation and by immunoperoxidase staining of intracellular cytokeratins.

Culture of umbilical vein endothelial cells

Human umbilical cords were collected immediately after parturition, the umbilical vein was cannulated and the vessel was perfused with heparinized Medium 199 (Sigma). The distal end of the cord was cross-clamped, the vein was filled with 0·25% (w/v) collagenase (Sigma) in Medium 199 and the proximal end was clamped. After incubation at room temperature for 30 min the clamps were removed and the contents of the vein were recovered by perfusion with Medium 199 supplemented with 10% (v/v) foetal calf serum. The cells were collected by centrifugation at 400 *g* for 10 min and the pellet was suspended in 10 ml of Medium 199 supplemented with 20% foetal calf serum, endothelial cell growth factor (100 μg/ml; Sigma) and heparin (100 μg/ml; Sigma). The cells derived from a single cord were cultured in two 25 cm^2 flasks pre-coated for primary cell cultures (Primaria; Falcon) and the developing monolayers were divided as necessary by trypsinization. Samples of the cells cultured from each specimen were propagated on glass slides and were characterized by morphological investigation and, after ethanol fixation, by immunofluorescence staining of intracellular Factor VIII (anti-Human Factor VIII. FITC; ICN Immunobiologicals, High Wycombe, Bucks, U.K.)

Spleen cell preparation

Human spleen samples were recovered during cadavaric organ donation and were stored in heparinized RPMI-1640 medium at 4° prior to processing. Splenocytes were teased from the tissue and were separated from erythrocytes by centrifugation over a Ficoll–metrizoate (Lymphoprep; Nycomed U.K., W. Midlands, U.K.) density gradient[17] for 25 min at 400 *g*. Mononuclear cells recovered from the interfacial band were washed twice by centrifugation and aliquots of 2×10^7 cells were suspended in complete medium containing 10% (v/v) DMSO prior to storage in liquid nitrogen.

Quantification of cell-surface antigen

The level of expression of class II MHC antigens on the surface of either resting or IFN-γ (Boehringer Mannheim, Lewes, East Sussex, U.K.)-treated cells was estimated by continuous flow microfluorimetry. Cells were suspended and washed in 'staining medium' consisting of RPMI-1640 medium supplemented with 1% (v/v) foetal calf serum and 0·01% (w/v) sodium azide (Sigma). A FITC-conjugated monoclonal antibody specific for the β-chain of all products of the HLA gene subregions DP, DQ and DR (CR3/43; Dako, High Wycombe, Bucks, U.K.) was added for 30 min and the cells were washed three times by centrifugation in staining medium at 4°. The cells were analysed using a FACscan (Becton-Dickinson) running FACscan Research Software (Becton-Dickinson) and the median fluorescence signal was calibrated by comparison with the signal generated using fluorescent microbeads (Flow Cytometry Standards Corporation, Research Triangle Park, NC) which were conjugated with either $1{\cdot}8 \times 10^6$, $4{\cdot}3 \times 10^5$, $1{\cdot}8 \times 10^5$ or $6{\cdot}4 \times 10^4$ FITC molecules per bead. The median level of class II MHC antigen expression was calculated in terms of the median number of FITC molecules, and hence anti-class II antibodies, bound to the cells.

Allostimulation of lymphocytes

Peripheral blood mononuclear cells (PBMC) were recovered from normal heparinized peripheral blood by centrifugation over Ficoll–metrizoate, and after washing twice by centrifugation were resuspended in complete medium at a concentration of 1×10^6 cells/ml.

Prior to mixing with PBMC the stimulator cells were irradiated; epithelial cells received 45 Gy, and endothelial and splenic cells received 25 Gy of X-irradiation. Stimulator cells were titrated into the wells of 96-well plates in triplicate at doses of 1×10^5 cells/well, 5×10^4 cells/well, $2{\cdot}5 \times 10^4$ cells/well, $1{\cdot}25 \times 10^4$ cells/well, $6{\cdot}3 \times 10^3$ cells/well and 0 cells/well. Flat-bottomed 96-well plates (Nunc Plastics; Gibco Ltd, Paisley, Renfrewshire, U.K.) were used for titrations of adherent epithelial cells and endothelial cells and round-bottomed plates

(Nunc Plastics) were used for titrations of non-adherent splenic cells. 1×10^5 PBMC were added to each well in a total volume of 200 μl and the plates were incubated for 5 days at 37° in a humid atmosphere of air containing 5% CO^2.

After incubation for this period each well was pulsed with 1 μCi of [^{3}H]thymidine (TRA-61; Amersham International PLc, Amersham, Bucks, U.K.) and after incubation for a further 12 hr the plates were harvested (Automash 2000; Dynatech, Billinghurst, Sussex, U.K.) onto glass-fibre filters (Whatman, Maidstone, Kent, U.K.) and the amount of DNA synthesis in each well was estimated by β-counting (Tricarb 4000; Packard, Caversham, Berks, U.K.).

Bulk mixed lymphocyte reactions (MLR) were established by mixing 5×10^6 PBMC with 5×10^6 irradiated splenic cells in 10 ml of complete medium in vertical 25 cm^2 tissue culture flasks.

Cytotoxicity assays

Approximately 1×10^6 renal epithelial cells were resuspended in 100 μl of RPMI-1640 medium containing 200 μCi of $Na_2{}^{51}CrO_4$ (600 mCi/mg/Cr; CJS-4; Amersham International) for 90 min. The cells were then washed three times by centrifugation in complete medium and adjusted to $2{\cdot}5 \times 10^4$ cells/ml. One-hundred microlitres of these target cells were mixed with 100 μl of effector lymphoid cells at effector:target ratios of 50:1, 25:1, and 12·5:1 in the wells of round-bottomed 96-well plates. Maximal release of ^{51}Cr was determined after two freeze-thaw cycles of the target cells and spontaneous release was measured after incubation of the target cells in the absence of effector cells. After incubation for 4 hr the plates were centrifuged at 200 *g* for 5 min to pellet the cells and 100 μl of medium were recovered from each well for gamma spectrometry (LKB-Wallac Clinigamma 1272; Pharmacia-LKB, Milton Keynes, Bucks, U.K.). The spontaneous release of ^{51}Cr did not exceed 9% of the maximal release. The percentage specific release of ^{51}Cr due to the action of cytotoxic effector cells was calculated by use of the following equation:[18]

$$\% \text{ specific } {}^{51}\text{Cr release} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \times 100.$$

RESULTS

After a minimum of three serial passages the renal cells were stained immunocytochemically to demonstrate the presence of intracellular cytokeratin (Fig. 1). At this stage 100% of the cells were shown to contain cytokeratin whilst cells treated with non-specific antibodies showed no staining after immunocytochemical development. Antisera specific for Factor VIII did not bind to the cultured renal cells. Cultured HUVEC showed a uniform positive fluorescence when stained with anti-Factor VIII. FITC antibodies.

Before stimulation with IFN-γ none of the cultured renal epithelial cells was labelled significantly by incubation with the anti-class II antibody and on FACS analysis showed a median fluorescence signal, attributable to cellular autofluorescence, equivalent to $4{\cdot}4 \times 10^4$ FITC molecules. The results in Fig. 2 were corrected for autofluorescence and are expressed as the median number of FITC molecules per cell; these values are not equal to, but are in direct proportion to, the number of class II molecules on the cells. After incubation with IFN-γ at a concentration of 250 U/ml the labelled cells showed an increase in median fluorescence, and hence in class II antigen expression, which peaked after 4 days at a value equivalent to $2{\cdot}4 \times 10^5$ FITC molecules per cell (Fig. 2); IFN-γ-treated epithelial cells were routinely harvested after 4 days for use in lympho-proliferative assays. Cultured HUVEC showed no significant expression of class II antigens in the absence of stimulation but after addition of IFN-γ the expression of these molecules was upregulated with similar kinetics to those observed for renal epithelial cells. Non-stimulated class II MHC antigen-expressing splenocytes showed a median fluorescence equivalent to $1{\cdot}9 \times 10^5$ FITC molecules per cell (Fig. 2).

Addition of PBMC to irradiated allogeneic splenic cells resulted in a variable lympho-proliferative response; positive reactions showed maximal proliferation in assays containing a stimulator:responder cell ratio of 1:1 and demonstrated a decreasing proliferation in assays containing a reducing stimulator:responder cell ratio (Fig. 3a). Mixtures of PBMC and irradiated HUVEC showed a smaller maximal proliferation

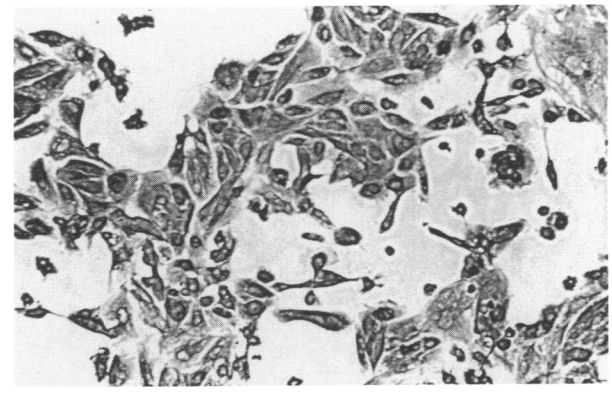

Figure 1. Primary renal epithelial cells stained by immunoperoxidase to show intracellular cytokeratin. The filamentous distribution of the protein is clearly visible (×240).

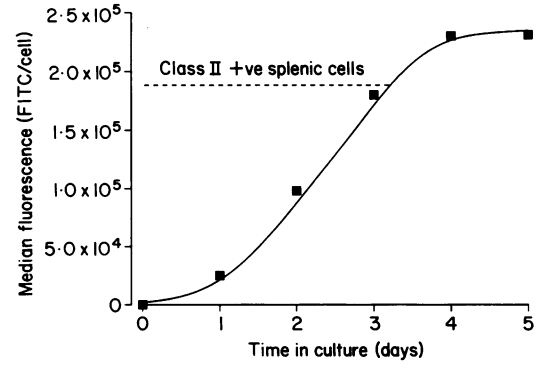

Figure 2. Time-course for the up-regulation of class II MHC antigens on the surface of renal epithelial cells cultured in the presence of 250 U/ml IFN-γ. The data are expressed in terms of the median number of FITC molecules bound to each cell after labelling with a FITC-conjugated anti-class II MHC antigen-specific monoclonal antibody; the fluorescence of class II MHC antigen-expressing splenocytes is shown for comparison. The absolute fluorescence values were determined by comparison with beads conjugated with a known number of FITC molecules.

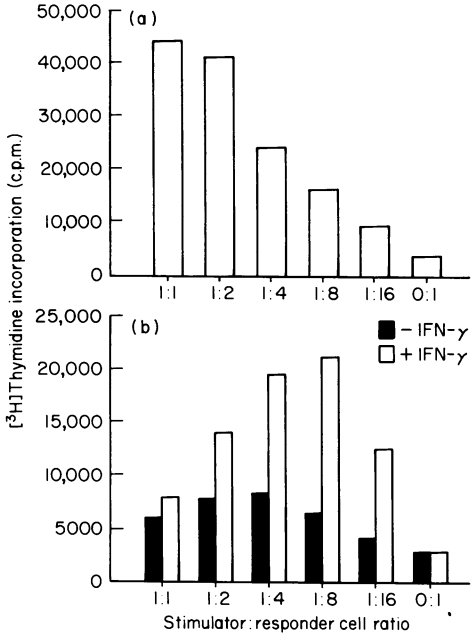

Figure 3. (a) Incorporation of [^{3}H]thymidine into PBMC stimulated by mixture with allogeneic spleen cells. (b) Incorporation of [^{3}H]thymidine into PBMC stimulated by mixture either with HUVEC which had been pretreated by incubation with 250 U/ml IFN-γ for 4 days or with non-pretreated HUVEC. For both assays the points represent the mean value of triplicate determinations; the data fell within 10% of the mean value for each point.

which occurred at a stimulator:responder cell ratio of between 1:4 and 1:8 (Fig. 3b). The use of HUVEC which had been previously exposed for 4 days to 250 U/ml of IFN-γ enhanced the lympho-proliferation but did not augment the response to levels routinely achieved in MLR.

Addition of PBMC either to unstimulated or to IFN-γ pretreated renal epithelial cells resulted in no significant lympho-proliferation at any of the tested stimulator:responder cell ratios. Indeed, MLKC performed in the presence of decreasing numbers of IFN-γ-treated epithelial cells showed a concomitant small increase in spontaneous lympho-proliferation (Table 1). Furthermore, proliferation in response to MLKC did not occur in the presence of either or both 1 μg/ml indomethacin (Sigma) and 20 U/ml rIL-1 (Boehringer Mannheim) or in assays supplemented with 3 μg/ml 5-fluorodeoxyuridine (FUdR; Sigma) at the time of pulsing with [^{3}H]thymidine (Table 1). However, culture in the presence of 25 U/ml rIL-2 (Boehringer Mannheim) stimulated the proliferation of PBMC mixed with IFN-γ-stimulated renal epithelial cells (Fig. 4); in this system maximal proliferation was achieved at a stimulator:responder cell ratio of between 1:4 and 1:16.

Bulk MLKC were established in horizontal 25 cm^2 flasks by mixing $1{\cdot}25 \times 10^6$ irradiated, IFN-γ-pretreated renal epithelial cells with 1×10^7 PBMC in the presence of rIL-2. After 5 days the proliferating lymphoid cell population was tested for specific cytotoxicity against both the renal cell line used to stimulate the bulk culture and a third-party renal epithelial cell line and was found to be equally lytic to both targets (Fig. 5). The phenotypic composition of the culture lymphoid cells was determined by continuous flow microfluorimetry after staining with fluorochrome-conjugated monoclonal antibodies specific for CD4,

Table 1. Effect of adding drugs and IL-1 to MLKC assays

Number of IFN-γ-treated epithelial cells	Treatment	[^{3}H]thymidine incorporation (c.p.m.) (mean of triplicate determinations)
0	—	3683
0	PHA*	144,327
0	Indomethacin†	2909
0	IL-1‡	3875
0	FUdR§	2170
$2{\cdot}5 \times 10^4$	—	1620
$1{\cdot}3 \times 10^4$	—	2230
$0{\cdot}6 \times 10^4$	—	3520
$2{\cdot}5 \times 10^4$	Indomethacin	3860
$2{\cdot}5 \times 10^4$	IL-1	4240
$2{\cdot}5 \times 10^4$	Indomethacin + IL-1	2990
$2{\cdot}5 \times 10^4$	FUdR	3670

* Positive control—1% (v/v) PHA (HA-15; Wellcome Diagnostics).
† Indomethacin at 1 μg/ml
‡ IL-1 at 20U/ml.
§ 5-Fuorodexoyruidine at 3 μg/ml.

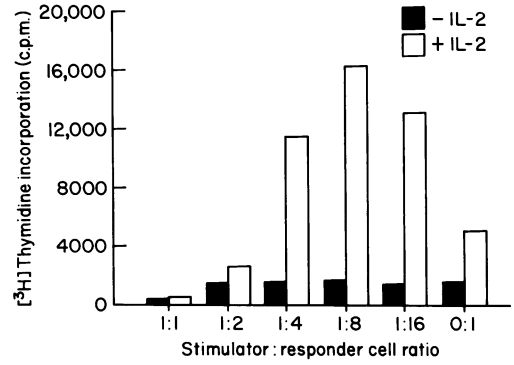

Figure 4. Incorporation of [^{3}H]thymidine by PBMC mixed with IFN-γ pretreated renal epithelial cell in the presence or the absence of 25 U/ml IL-2. For both assays the points represent the mean value of triplicate determinations; the data fell within 10% of the mean value for each point.

CD8 and CD16 (Becton-Dickinson). The three cell populations examined showed similar CD4:CD8 cell ratios to the preculture values (ranging from 1·15:1 to 1·7:1) but the proportion of CD16 positive cells had increased from between 9% and 16% to between 26% and 38% of the total lymphoid cell population which was defined on the basis of its forward and 90° light-scatter characteristics.

Stimulation of PBMC in bulk MLR culture for 7 days yielded an allospecific T-cell line which was then used in MLKC reactions. The T-cell line was mixed in the absence of exogenous cytokines with IFN-γ-treated renal epithelial cells either from the same donor as the splenic stimulator cells used in the MLR or from a third-party donor. In these experiments lympho-proliferation was observed in assays stimulated using the

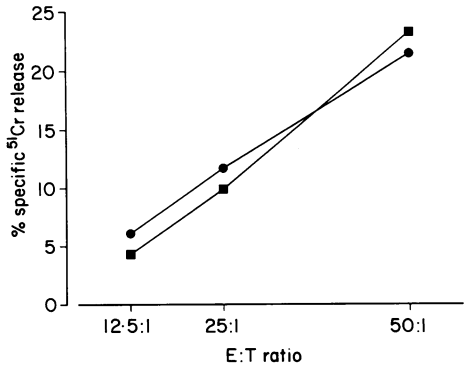

Figure 5. Specific cytotoxicity of a cell line generated by IL-2-stimulated MLKC towards either the epithelial cell line used to stimulate the MLKC (■) or towards a third-party renal epithelial cell line (●). For both assays the points represent the mean value of triplicate determinations; the data fell within 10% of the mean value for each point.

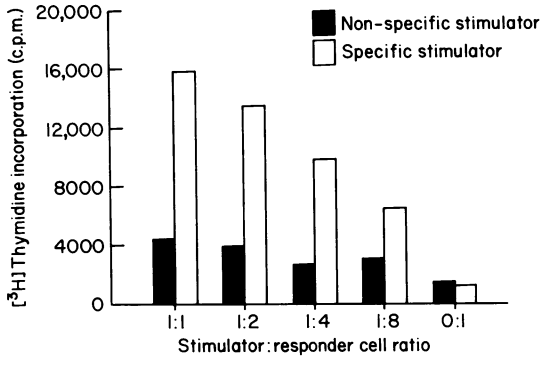

Figure 6. Incorporation of [^{3}H]thymidine by lymphoid cells stimulated for 7 days with irradiated splenocytes and then mixed with IFN-γ-treated renal epithelial cells either from the same donor as the spleen sample or a third-party donor. For both assays the points represent the mean value of triplicate determinations; the data fell within 10% of the mean value for each point.

specific renal cell line but no proliferation was observed after addition of third-party renal cells (Fig. 6).

DISCUSSION

Purified lines of human renal epithelial cells were propagated in order to ascertain their capacity to stimulate proliferation of allogeneic lymphocytes. All of the cultured cells showed the presence of intracellular cytokeratin; these prekeratin-like molecules are characteristic of epithelial cells.[19] Factor VIII, which is characteristic of endothelial cells, was not identified in any of the long-term cultured renal cells. The epithelial cell lines were derived from a variety of human renal specimens obtained at nephrectomy for renal cell carcinoma and end-stage allograft rejection. It is likely, therefore, that some of the epithelial cells present in the original tissue were stimulated *in vivo* to express class II MHC antigens. However, after a minimum of three serial passages the homogeneous epithelial cell populations (Fig. 1) expressed no detectable surface class II MHC antigens. This has previously been reported for renal epithelial cells cultured from rejected allografts[20] and is consistent with the rapid down-regulation in class II antigen expression which has been observed for renal cell cultures deprived of IFN-γ.[8] After the addition of IFN-γ to epithelial cell cultures the median number of class II MHC antigens present on the surface of the cells increased rapidly. Cultured HUVEC were uniformly labelled by anti-Factor VIII antibodies and showed a similar up-regulation in class II MHC antigen expression on addition of INF-γ. After stimulation by IFN-γ for 4 days the median number of class II MHC molecules per epithelial cell exceeded the number on resting class II antigen-expressing splenic cells.

Unlike renal epithelial cells both splenic cells and IFN-γ-treated and untreated HUVEC stimulated the proliferation of allogeneic lymphocytes (Fig. 3a, b). HUVEC stimulated maximal lympho-proliferation at a stimulator:responder ratio which was lower than that required for maximal MLR proliferation. Although IFN-γ-treated HUVEC were more efficient at stimulating lympho-proliferation than non-treated HUVEC, the non-treated cells stimulated significant proliferation in the absence of detectable class II antigen expression. This finding has been observed previously[6,7,21] and may demonstrate either that a few activated lymphocytes in the PBMC preparation can produce sufficient cytokine to up-regulate expression of class II on the HUVEC cells or that unmeasurably low levels of constitutive class II MHC antigen expression on HUVEC are sufficient to stimulate the proliferation of allogeneic lymphocytes. Although it is difficult to quantify antigen density on a cell surface it is clear that the degree of proliferation of alloreactive lymphocytes is not simply a function of the number of class II MHC antigens on stimulator cells. Indeed, presentation of only 200–300 peptide/class II complexes by APC may be sufficient to stimulate maximal proliferation of specific T lymphocytes.[22] The conflicting results of MLKC assays reported previously may be explained in terms of varying purity of the renal cell lines used in lympho-proliferative assays. For example Roth *et al.*[12] reported the presence of human MLKC reactivity but used kidney cortical cells which had not been cultured prior to establishment of the assay; it is possible that some APC may have contaminated these cultures.

A series of experiments was conducted in order to determine whether apparent failure of lympho-proliferation in the MLKC reaction was caused by the epithelial cells either producing immunosuppressive factors, producing non-radiolabelled thymidine or failing to produce co-stimulatory factors (Table 1). Assays were established in the presence of indomethacin, which blocks production of immunosuppressive prostaglandins by inhibiting cyclo-oxygenase, and of FUdR, which prevents *de novo* synthesis of non-raidolabelled thymidine by inhibiting thymidine synthetase.[23] Neither of these agents allowed significant incorporation of [^{3}H]thymidine to occur during the MLKC reaction. IL-1 is considered to be an important co-stimulatory factor which can play a role in initiating lymphocyte activation;[24] the addition of exogenous IL-1 in the presence or absence of indomethacin had no effect on lympho-proliferation.

Addition of exogenous IL-2 to MLKC reactions stimulated the proliferation of allogeneic lymphocytes (Fig. 4). The enhanced proliferation of IL-2-stimulated lymphocytes over non-stimulated lymphocytes in the absence of epithelial cells indicates the presence of activated lymphocytes in the normal cultured PBMC population; this spontaneous proliferation

appears inhibited in the presence of epithelial cells at a stimulator to responder cell ratio of above 1:2.

A number of reports have suggested that certain class II MHC antigen-expressing cells fail to stimulate the proliferation of antigen-specific lymphocytes. Such reports include studies of class II molecules expressed by planar lipid membranes, fibroblasts and chemically modified splenic cells.[7,25,26] More recent studies using tansgenic mice expressing additional MHC molecules on pancreatic islet cells[27,28] or on a variety of parenchymal cells, including those of the kidney,[29] have also demonstrated either *in vitro* or *in vivo* lymphocyte unresponsiveness. Several of there reports, however, have shown that the lymphocyte unresponsiveness induced by potentially immunogenic MHC molecules expressed by non-antigen-presenting cells (APC) can be overcome by the addition of exogenous IL-2.[25,26] It seems likely that class II MHC antigen-expressing non-APC are unable to provide a co-stimulatory signal which is essential for the production of sufficient IL-2 to allow lympho-proliferation. This may be the explanation for the different MLKC results observed in the presence and absence of exogenous IL-2.

The establishment of bulk MLKC in the presence of IL-2 allowed the generation of a line of lymphoid cells which showed similar CD4:CD8 ratios to those of unstimulated PBMC. However, the line did show an increase in the proportion of lymphoid cells bearing the natural killer (NK) cell-associated Fc receptor for IgG (CD16). The cell line exhibited cytotoxicity both for the epithelial cells used to stimulate the bulk MLKC and for a third-party renal epithelial cell line (Fig. 5). These findings are similar to those observed for the lysis of renal epithelial cells by lymphokine-activated killer cells generated from PBMC by culture with IL-2[15,30] and may correlate with the enhanced proportion of CD16-positive cells in the cell line produced by IL-2 stimulated MLKC.

The secondary proliferation of MLR-activated lymphocytes in the presence of IFN-γ-treated renal epithelial cells derived from the same donor as the splenic cells used for the initial MLR (Fig. 6) is indicative of a specific response to alloantigens expressed by both the epithelial and the splenic cells and is not a feeder-cell effect as third-party renal cells were not stimulatory. It appears that lymphocytes which have been previously activated by interaction with alloantigens presented by APC are able to proliferate further in response to similar antigens expressed by renal epithelial cells without the requirement for exogenous IL-2 observed for primary MLKC reactions.

The results presented in this report demonstrate that class II MHC antigen-expressing renal epithelial cells are unable directly to stimulate the proliferation of resting lymphocytes. However, in the intragraft situation it is likely that high local concentrations of IL-2 are present and that many of the graft infiltrating allospecific lymphocytes will be primed by previous contact with donor APC such as vascular endothelial cells or dendritic cells. Consequently, expression of class II MHC antigens by epithelial cells within a renal allograft may still render such cells immunogenic and able to play a direct role in the lympocyte-mediated intragraft rejection process.

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REFERENCES

1. Lechler R.I., Lombardi G., Batchelor J.R., Reinsmoen N. & Bach F.H. (1990) The molecular basis of alloreactivity. *Immunol. Today*, **11**, 83.
2. Hall B.M., Bishop G.A., Duggin G.G., Horvath J.S., Philips J. & Tiller D.J. (1984) Increased expression of HLA DR antigens on renal tubular cells in renal transplants: relevance to the rejection response. *Lancet*, **ii**, 247.
3. Rose M.L., Coles M.I., Griffin R.J., Pomerance A. & Yacoub M.H. (1986) Expression of Class I and Class II major histocompatibility antigens in normal and transplanted human heart. *Transplantation*, **41**, 776.
4. Kuntz-Crow M. & Kunkel H.G. (1982) Human dendritic cells: major stimulators of the autologous and allogeneic mixed leukocyte reactions. *Clin. exp. Immunol.* **49**, 388.
5. Frohman M. & Cowing C. (1985) Presentation of antigen by B cells: functional dependence on radiation dose, interleukins, cellular activation and differential glycosylation. *J. Immunol.* **134**, 2269.
6. Hirschberg H., Evensen S.A., Henriksen T. & Thorsby E. (1975) The human mixed lymphocyte–endothelium culture interaction. *Transplantation*, **19**, 495.
7. Geppert T.D. & Lipsky P.E. (1985) Antigen presentation by interferon-gamma-treated endothelial cells and fibroblasts: Differential ability to function as antigen-presenting cells despite comparable Ia expression. *J. Immunol.* **135**, 3750.
8. Bishop G.A., Hall B.M., Suranyi M.G., Tiller D.J., Horvath J.S. & Duggin G.G. (1986) Expression of HLA antigens on renal tubular cells in culture. I. Evidence that mixed lymphocyte culture supernatants and gamma interferon increase both Class I and Class II HLA antigens. *Transplantation*, **42**, 671.
9. Halloran P.F., Wadgymar A. & Autenreid P. (1986) The regulation of expression of major histocompatibility complex products. *Transplantation*, **41**, 413.
10. Romaniuk A., Prop J., Peterson A.H., Wildevuur C.R.H. & Nieuwenhuis P. (1987) Expression of Class II major histocompatibility antigens by bronchial epithelium in rat lung allografts. *Transplantation*, **44**, 209.
11. Esquenazi V., Fuller L., Paedo V., Roth D., Milgrom M. & Miller J. (1987) *In vivo* and *in vitro* induction of Class II molecules on canine renal cells and their effect on the mixed lymphocyte kidney cell culture. *Transplantation*, **44**, 680.
12. Roth D., Fuller L., Esquenazi V., Kyriakides G.K., Pardo V. & Miller J. (1985) The biologic significance of the mixed lymphocyte kidney culture in humans. *Transplantation*, **40**, 376.
13. Vegt P.A., Van der Linden C.J., Daemen A.J.J., Jeekel J. & Buurman W.A. (1982) Lymphocyte stimulation by canine kidney cells. *Transplantation*, **34**, 134.
14. Bishop G.A., Waugh J.A. & Hall B.M. (1988) Expression of HLA antigens on renal tubular cells in culture. II. Effect of increased HLA antigen expression on tubular cell stimulation of lymphocyte activation and on their vulnerability to cell-mediated lysis. *Transplantation*, **46**, 303.
15. Kirby J.A., Forsythe J.L.R., Proud G. & Taylor R.M.R. (1989) Renal allograft rejection: possible involvement of lymphokine-activated killer cells. *Immunology*, **67**, 62.
16. Giblert S.F. & Migeon B.R. (1975) D-Valine as a selective agent for normal human and rodent epithelial cells in culture. *Cell*, **5**, 11.
17. Boyum, A. (1968) A one-stage procedure for the isolation of granulocytes and lymphocytes from human blood: general sedimentation properties of white blood cells in a 1 × g gravity field. *Scand. J. clin. Lab. Invest.* **21**, 97 (Suppl).
18. Brunner K.T., Mauel J., Cerrotini J.-C. & Chapuis B. (1968)

Quantitative assay of immune lymphoid cells on 51-Chromium labelled allogeneic targets *in vitro*: inhibition by isoantibody and by drugs. *Immunology*, **14,** 181.

19. FRANKE W.W., WEBER K., OSBORN M., SCHMID E. & FREUDENSTEIN C. (1978) Antibody to prekeratin. Decoration of tonofilament-like arrays in various cells of epithelial character. *Exp. Cell Res.* **116,** 429.
20. KIRBY J.A., FORSYTHE J.L.R., SIMM A., PROUD G. & TAYLOR R.M.R. (1989b) Renal allograft rejection: protection of renal tubular epithelial cells from lymphokine activated killer cell mediated lysis by pretreatment with cytokines. *Nephrol. Dial. Transplant.* **4,** 824.
21. POBER J., COLLINGS M., GIMBRONE M. JR., COTRAN R., GITLIN J., FIERS W., CLAYBERGER C., KRENSKY A., BURAKOFF S. & REISS C. (1983) Lymphocytes recognise human vascular endothelial and dermal fibroblast Ia antigens induced by recombinant immune interferon. *Nature*, **305,** 726.
22. HARDING C.V. & UNANUE E.R. (1990) Quantitation of antigen-presenting cell MHC class II/peptide complexes necessary for T-Cell stimulation. *Nature*, **346,** 574.
23. WHITE A., HANDLER P., SMITH E.L., HILL R.C. & LEHMAN I.R. (1978) *Principles of Biochemistry*, 6th edn, p. 775. McGraw Hill, New York.
24. SCALA G. & OPPENHEIM J.J. (1983) Antigen presentation by human monocytes: evidence for stimulant processing and requirement for interleukin 1. *J. Immunol.* **131,** 1160.
25. QUILL H. & SCHWARTZ R.H. (1987) Stimulation of nornal inducer T cell clones with antigen presented by purified Ia molecules in planar lipid membranes: specific induction of a long-lived state of proliferative unresponsiveness. *J. Immunol*, **138,** 1987.
26. JENKINS M.K. & SCHWARTZ R.H. (1987) Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness *in vitro* and *in vivo*. *J. exp. Med.* **165,** 302.
27. MARKMANN J., LO D., NAJI A., PALMITER R.D., BRINSTER R.L. & HEBER-KATZ E. (1988) Antigen presenting function of class II MHC expressing pancreatic beta cells. *Nature*, **336,** 476.
28. MURPHY K.M., WEAVER, C.T., ELISH M., ALLEN P.M. & LOH D.Y. (1989) Peripheral tolerance to allogeneic class II histocompatibility antigens in transgenic mice: evidence against clonal deletion. *PNAS U.S.A.*, **96,** 10034.
29. MORAHAN G., BRENNAN F.E., BHARTHAL P.S., ALLISON J., COX K.O. & MILLER J.F.A.P. (1989) Expression in transgenic mice of class I histocompatibility antigen controlled by metallothionein promoter. *PNAS U.S.A.*, **86,** 3782.
30. MILTENBURG A.M.M., MEIJER-PAAPE M.E., DEHA M.R. & PAUL L.C. (1988) Lymphokine activated killer cells lyse human renal cancer cell lines and cultured normal kidney cells. *Immunology*, **63,** 729.