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T-cell anergy: a consequence of interaction between T cells and allogeneic rat renal epithelial cells

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Abstract In order to study the immunogenicity of parenchymal cells within an allograft, renal tubular cells were propagated from both PVG and DA strain rats. These cells were induced to express class II major histocompatibility (MHC) antigens by stimulation for 4 days with interferon-gamma (IFN-γ). It was found that resting lymphoid cells derived from Lewis rats responded vigorously after stimulation with irradiated splenic cells from PVG rats. However, stimulation with renal cells from PVG rats did not result in interleukin (IL-2) production or lymphoproliferation. Furthermore, lymphocytes from this mixture failed to respond to secondary stimulation by PVG splenic cells; lymphocytes primed by mixture with DA renal cells responded normally to secondary stimulation by PVG splenic cells. These results indicate that renal epithelial cells can specifically anergise allogeneic lymphocytes.

Key words Anergy · T cell · Kidney Tubular epithelium

Introduction

It is known that the interaction between class II major histocompatibility (MHC) antigens on donor antigen-presenting cells and the T cell receptor on allospecific recipient CD4+ lymphocytes is of crucial importance to the development of allograft rejection [1]. Proinflammatory cytokines such as interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) are produced during allograft rejection. These cytokines are thought to lead to the observed induction of class II MHC antigens on parenchymal cells within the graft [2]. The consequences of the interaction between class II MHC antigen-expressing parenchymal cells and resting allospecific lymphocytes are not clearly understood. It is possible that such an interaction may result in T-cell activation. However, it is also possible that interaction between lymphocytes and class II MHC antigens which are "inappropriately" presented by parenchymal cells may produce stable T-cell anergy [3, 4].

In this report the results of mixing class II MHC antigen-expressing rat renal tubular epithelial cells and allogeneic peripheral blood lymphocytes are examined.

Materials and methods

Animals

Inbred male PVG ($RT1^c$), DA ($RT1^a$) and Lewis ($RT1^l$) rats (200–300 g; B & K Universal, Hull, UK) were maintained in accordance with the UK Animals (Scientific Procedures) Act, 1986.

Renal cell preparation and culture

Cortical tissue was dissected from rat kidneys, chopped and pressed through a 250-μm mesh [5]. Tubular fragments were separated from

the disaggregated tissue by retention on a 100-μm mesh. These were cultured on fibronectin (Sigma, Dorset, UK)-coated 25-cm^2 flasks (Falcon, Becton-Dickinson, Oxon, UK) in D-valine containing minimal essential medium (Gibco, Renfrewshire, UK) supplemented with 10% fetal bovine serum, HEPES buffer (10×10^{-3} M), 2-mercaptoethanol (5×10^{-5} M), insulin-transferrin-selenium (Sigma), hydrocortisone (5×10^{-8} M; Sigma), tri-iodothyronine (3×10^{-8} M; Sigma), ampicillin and streptomycin (Sigma). Some cells were treated with 250 U rat IFN-γ/ml (Holland Biotechnology, Leiden, the Netherlands). Cells were used between passes 2 and 3.

Allostimulation of lymphocytes

A constant number (5×10^4) of adherent cell-depleted, peripheral blood-derived mononuclear cells (PBMC) were mixed with irradiated splenic mononuclear cells or renal epithelial cells at a range of responder to stimulator cell ratios. Assays were performed in RPMI 1640 medium supplemented with 10% fetal calf serum, 10×10^{-3} M HEPES and antibiotics. After incubation for 5 days, each culture was pulsed with 1 μCi of [^{3}H]-thymidine (TRA 61; Amersham International, Bucks, UK) for 6 h prior to harvesting onto glass-fibre filters and β-scintillation counting.

Interleukin-2 bioassay

The supernatant from mixed cultures containing lymphoid and allogeneic renal epithelial cells was assayed for the presence of interleukin 2 (IL-2) by use of the IL-2-dependent cell line CTLL-2 (ECACC Porton Down, Wiltshire, UK). The proliferation of CTLL-2 was assessed by measuring the incorporation of [^{3}H]-thymidine as described above.

Development and assay of lymphocyte anergy

Confluent renal epithelial cells cultured from PVG and from DA rats were treated with IFN-γ for 4 days. After 5 days of incubation, 5×10^6 PBMC from a Lewis rat were added to each culture for a period of 4 days. The mononuclear cells were then recovered and assessed for their ability to proliferate when stimulated with splenic cells from a PVG rat for 5 days as described above.

Results

Immunocytochemical staining showed that more than 90% of the cultured renal cells expressed cytoplasmic cytokeratins. Treatment of these cells for 4 days with IFN-γ resulted in the induction of class II MHC antigens on the surface of more than 90% of the cells. Flow microfluorimetry showed that the median fluorescence of FITC-conjugated anti-class II antibody-stained cells increased from a mean background of 34 to 795 units after treatment with IFN-γ.

The result of a 5-day mixed leukocyte culture between Lewis responder cells and PVG stimulator cells is shown in Fig. 1. The titration produced a maximal lymphoproliferative response at a responder to stimulator cell ratio of 1:1. Control cultures showed little proliferation after autologous stimulation.

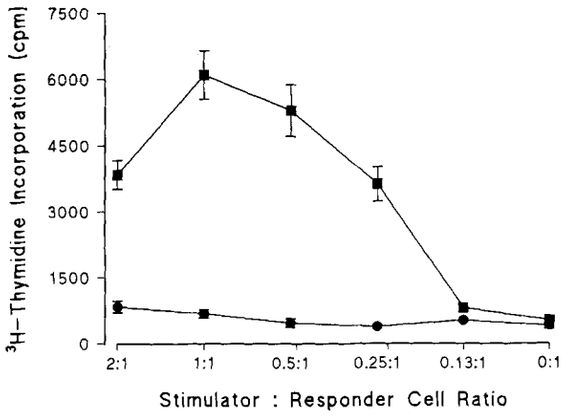

Fig. 1 Lymphoproliferation generated in a representative 5-day mixed leukocyte reaction (■ Lewis responder cells PVG splenic stimulator cells; ● Lewis responder cells, Lewis splenic stimulator cells). The *points* represent the mean of triplicate determinations; the *error bars* show the standard deviation of the data

Table 1 Five-day mixed leukocyte-kidney cell culture results

Stimulation of 5×10^4 LEW responder cells	[^{3}H]-Thymidine incorporation (cpm)[a]
None	843
5×10^4 IFN-γ-treated PVG renal cells	539
2.5×10^4 IFN-γ-treated PVG renal cells	584
1.3×10^4 IFN-γ-treated PVG renal cells	753
0.6×10^4 IFN-γ-treated PVG renal cells	694
5×10^4 PVG splenic cells	6038
1 μg/ml phytohaemagglutinin	43648

[a] Mean of triplicate determinations

The results in Table 1 show the absence of significant lymphoproliferation after mixing Lewis responder cells with varying numbers of IFN-γ-pretreated PVG renal cells. The proliferative response generated in a mixed leukocyte culture and in a phytohaemagglutinin (PHA; Sigma) mitogenesis assay are shown for comparison. The CTLL-2 bioassay did not indicate the presence of a significant concentration of IL-2 at any time during the assay.

Figure 2 shows the results from an assay of lymphoproliferation generated by secondary mixed leukocyte culture with PVG splenic cells after priming Lewis lymphocytes by co-culture with IFN-γ-pretreated PVG or DA renal cells. The data show that Lewis lymphocytes are able to respond to PVG splenic stimulator cells after priming with DA renal cells but that the response is not

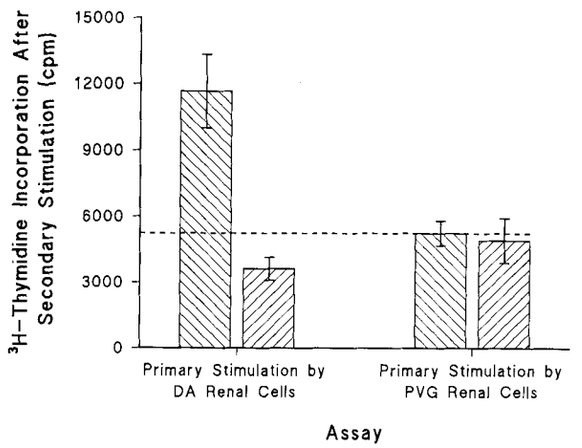

Fig. 2 Demonstration of allospecific hyporeactivity of Lewis rat T cells (▨ secondary stimulation by PVG splenic cells, ▨ secondary stimulation by Lewis splenic cells). The *bars* represent the mean of triplicate determinations; the *error bars* show the standard deviation of the data

significantly different from background after priming with PVG renal cells.

Discussion

The population of cultured renal cells showed morphological and biochemical characteristics of epithelial cells [6]. The induction of class II MHC antigens by treatment with IFN-γ was similar to that reported for cultured human tubular epithelial cells [7, 8].

The Lewis responder and PVG splenic stimulator cell combination produced a specific mixed leukocyte response. However, the combination of Lewis responder cells with class II MHC antigen-expressing renal epithelial cells did not generate significant concentrations of IL-2 or lymphoproliferation. It has been reported previously that human renal epithelial cells fail to stimulate the proliferation of allogeneic lymphocytes in mixed culture [9]. The failure of specific lymphoproliferation in this system may reflect an inability of renal epithelial cells to provide the co-stimulatory signals that are necessary before IL-2 generation can occur [4].

The lymphocytes not only failed to respond to allogeneic renal cells but subsequently failed to respond to stimulation by splenic cells syngeneic with the renal cells. This hyporeactivity was antigen-specific, as normal proliferation was observed if the renal cells used for the priming reaction were from a third-party rat strain. Previous reports have demonstrated similar findings using a human system [10] and alloreactive rat T-cell lines [11].

It has been proposed that T-cell receptor engagement with a target MHC-peptide complex in the absence of appropriate co-stimulation results in a state of specific T-cell anergy [3]. The results presented in this report are entirely consistent with this model and indicate that the class II MHC antigens expressed on parenchymal cells during graft rejection may have an immunoregulatory function.

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