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Profile of cytokine production in mixed kidney lymphocyte culture

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Abstract Kidney cells are an important source of immunoregulatory molecules that regulate cell-to-cell interactions, which is the key step in the generation of the immunoresponse to alloantigens. In this study we identified the cytokines that are produced by both lymphoid cells and kidney cells when coincubated in mixed kidney lymphocyte cultures (MKLC). The capacity of kidney cells to stimulate the proliferation of effector allogeneic lymphocytes was assayed by incubating irradiated kidney cells and lymphocyte. The cytokine secretion profile in MKLC was investigated by incubating monolayers of kidney cells with effector peripheral blood mononuclear cells (PBMC). The culture supernatants were harvested on days 1, 2, 3, 4, 5, 6, 7, and 8 and assayed for IL-1β, IL-2, IL-6, and TNF alpha using an ELISA. Kidney cells, in comparison to PBMC stimulator cells were poor stimulators of the allo-proliferation even when HLA expression was increased by IFN gamma treatment. Compared to lymphocyte or kidney cells incubated alone, MKLC induced a considerable stimulation of cytokine production. This increase in cytokine production was observed essentially for IL-2 and IL-6 (at day 3, a 10-fold increase in IL-2 and a 5-fold increase in IL-6). This study provided evidence that target kidney cells and effector lymphocyte interactions generate a number of cytokines such as IL-1β, IL-2, IL-6, or TNF alpha. These cytokines are known to modulate allo-proliferation and generation of cytotoxic T lymphocytes (CTL).

Key words Kidney cells
Lymphocytes · Cytokines

Introduction

In the allograft rejection model, lymphoid cells are viewed as stimulators of allospecific cytotoxic T lymphocytes (CTL), and parenchymal cells primarily as targets for effector CTL. However, the parenchymal cells can potentially participate in the afferent phase of the alloreactive response. These cells are an important source of immunoregulatory molecules that regulate cell-to-cell interactions, which is the key step in the generation of the immune response to alloantigens. In this study we identified the cytokines that are produced by both lymphoid cells and kidney cells when coincubated in mixed kidney lymphocyte cultures (MKLC).

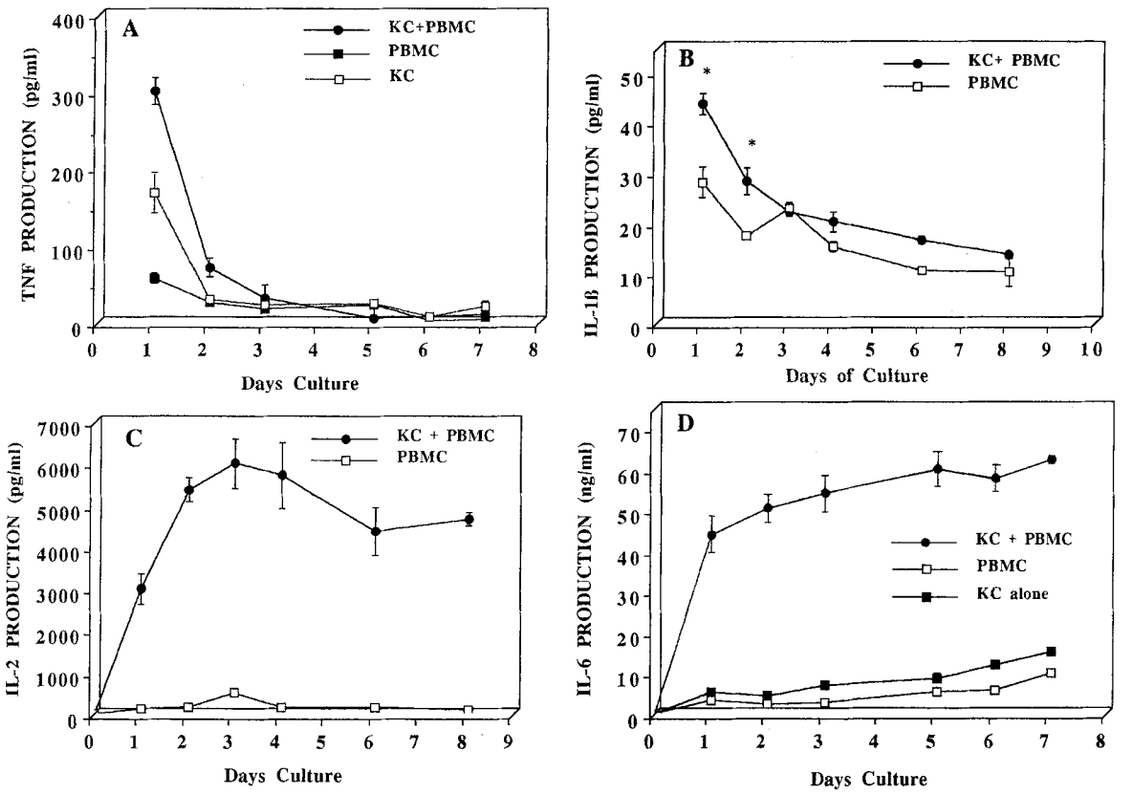

Fig. 1 A–D The profile of the temporal appearance of cytokines in allogeneic mixed kidney lymphocyte cultures (MKLC). The cytokine secretion profile in MKLC was investigated by incubating monolayers of kidney cells with effector peripheral blood mononuclear cells (PBMC). The culture supernatants were harvested on days 1, 2, 3, 4, 5, 6, 7, and 8 and assayed for IL-1β, IL-2, IL-6, and TNF alpha. Data are the mean $\pm$ SD (*KC* kidney cells, *PBMC* peripheral blood mononuclear cells)

Materials and methods

Human kidney cells (KC) and peripheral blood mononuclear cells (PBMC) were isolated as previously reported [1]. MKLC contained 5×10^4 kidney cells and 2×10^5 responder allogeneic or autologus peripheral blood mononuclear cells (PBMC). Triplicates of MKLC were cultured in 24-well plates (Corning, N.Y.) in RPMI 1640 supplemented with 10% FBS (Hyclone, Utah), glutamine, hepes buffer, and antibiotics (Flow) at 37 °C in 5% CO_2. Supernatants were harvested on days 1, 2, 3, 4, 5, 6, and 7, and stored at -20 °C until assayed for cytokine content. IL-2 and TNF alpha were measured by ELISA (R&D Systems, Minn.), and IL-6 was measured by bioassay (MH60 cell line) and ELISA (Tosoh, Kanazawa). IL-1β was measured by an ELISA assay kit purchased from Ohtsuka (Osaka). To assess whether kidney cells induce the proliferation of allogeneic PBMC, 10^5 irradiated stimulator kidney cells or PBMC were incubated with 10^5 responder PBMC in one-way MKLC or mixed lymphocyte cultures (MLC). The proliferation of responder cells was assessed by thymidine uptake on days 1, 2, 3, 4, 5, and 7.

Results

Kidney cells, compared to PBMC stimulator cells, were poor stimulators of allogeneic proliferation (8566 ± 620 cpm vs. 60428 ± 1338 cpm on day 7), even when HLA expression was increased by IFN' gamma treatment (data not shown). Figure 1 shows the profile of the temporal appearance of cytokines in allogeneic MKLC. TNF alpha displayed a very rapid rise, decreasing in less than 48 h. TNF alpha was secreted both by PBMC and kidney cells but more efficiently by kidney cells. When both cells were coincubated, a significant increase (1.7-fold increase on day 1) in cytokine production was observed (Fig. 1 A). Kidney cells did not secrete IL-1β in culture supernatant as shown in Fig. 1 B. The profile of the appearance of IL-1β in MKLC was comparable to that of TNF alpha. There was a very early rise in less than 24 h, followed by a rapid decrease. IL-1β production was significantly enhanced only in the early phase of MKLC (1.5- and 1.6-fold increase on days 1 and

2, respectively). In the late phase of MKLC, the production of IL-1β was comparable by PBMC alone and MKLC (Fig. 1 B). Only PBMC secreted IL-2, kidney cells did not release this cytokine in culture supernatant. Stimulation of PBMC with allogeneic kidney cells resulted in a significant production of IL-2 compared with PBMC cultured alone (10-fold increase on day 3; Fig. 1 C). The production of IL-2 displayed a very rapid rise on day 2 with a plateau on days 3, 4, and 5 followed by a slight decrease in the late phase of MKLC. IL-6 was produced by both lymphocytes and kidney cells. The coincubation of these cells resulted in significant increase in IL-6 secretion (5-fold increase on day 3; Fig. 1 D). IL-6 was produced initially in high amounts and accumulated slowly in the culture supernatant.

Discussion

This study provided evidence that target kidney cell and effector lymphocyte interaction generates a number of cytokines such as IL-1β, IL-2, IL-6, or TNF. However, appearance and clearance kinetics are different from cytokine to cytokine. Our study adds further evidence to the finding that TNF alpha and IL-1 are the earliest mediators of inflammation and the immune response, as has been reported in vivo [2] and in vitro [3]. Although allogeneic kidney cells stimulated the production of IL-2 and IL-6 supporting the growth and proliferation of lymphocytes, they were very poor stimulators of lymphocyte proliferation in MKLC compared with PBMC stimulators. It is unlikely that a modest antigen-presentation activity of kidney cells may explain this finding, because an important increase in HLA DR expression by IFN gamma treatment did not improve PBMC proliferation in MKLC. This phenomen has been reported by Bishop [4] and is attributed to a consumption of the growth medium or a consumption of some lymphocyte growth factor other than IL-1. We favor the hypothesis that kidney cells secrete some counteracting mediators that downregulate T cell proliferation even in the presence of high amounts of IL-2 or IL-6.

The cytokines described in this study are known to modulate the generation of cytotoxic T cells. The identification of these cytokines in MKLC suggests that these cytokines may play a role in the development of the CTL response to kidney cell alloantigens. The use of neutralizing antibodies to the cellular receptor of these cytokines should determine whether these cytokines are required for CTL generation in MKLC. In conclusion, this study may provide insight into the qualitative and quantitative aspects of cytokine production in kidney graft rejection.

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