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Difference in cytokine production in acute and chronic rejection of rat lung allografts

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Abstract In Brown Norway to Lewis rat lung transplantation, short-term administration of cyclosporine produces permanent adoption of allografts; however, the adopted grafts show symptoms of chronic rejection. To clarify the difference in cytokine production in acute an chronic rejection of the allografts, an immunohistochemical study was performed. In acute rejection, positive cells for respective cytokines were observed in infiltrating cells, increasing in number as the days after transplantation passed, and reaching a maximum on the fifth day. The strongest reactivity was observed perivenously. In chronic rejection, TNF-α positive cells were observed in the perivascular and peribronchial regions, especially around class II positive epithelia. The number of positive cells was, however, less than that in the vascular phase of acute rejection. Few cells were positive for IL-1β, IFN-γ and, unexpectedly, for IL-4. These facts indicate the functional difference of infiltrating cells between acute and chronic rejection.

Key words Cytokine production · Immunohistochemistry · Lung transplantation

Introduction

Chronic rejection is one of the catastrophic complications in human lung transplantation. Although many studies on human allografts have been published, reports on chronic rejection in animal models have been limited. In Brown Norway (BN) to Lewis (LEW) rat lung transplantation, allografts are indefinitely accepted after administering a short course of cyclosporine A, and the allografts show symptoms of chronic rejection including cellular infiltration reminiscent of the vascular phase of acute rejection [1]. The cellular infiltrates in the accepted allografts decrease gradually, while the damaged airway begins to exhibit bronchiolitis obliterans-like features [2].

In this rat model, we demonstrated a higher ratio of CD4/CD8 and a lower ratio of ED2/ED1 in cyclosporine-treated accepted allografts compared with acutely rejected non-treated allografts [3]. These differences in the subpopulation of lymphocytes and macrophages suggested possible differences in immune response, especially cytokine production in the respective allografts. Moreover, most CD4-positive cells in accepted allografts were negative for CD45RC [4]. This fact suggested the predominance of Th2 cells in accepted allografts and the possible production of IL-4 [5].

In this study, we investigated whether cytokine production in acute and chronic rejection was different. An immunohistochemical examination was performed with antibodies to proinflammatory cytokines (TNF-α, IL-1β), Th1-associated cytokine (IFN-γ), and Th2-associated cytokine (IL-4).

Materials and methods

Male inbred BN ($RT1^n$) and LEW (RT^1) were obtained from Charles River, Japan. All animals were kept under specific pathogen-free conditions. The left lung was transplanted orthotopically according to the improved technique [6] of Marck. Allogeneic

lung transplantation was performed from BN to fully mismatched LEW. In the acute rejection group (AR), no treatment was carried out after transplantation. In the chronic rejection group (CR), cyclosporine A (25 mg/kg) was injected intramuscularly on days 2 and 3 after the operation.

Recipients were sacrificed on days 1, 3, and 5 after the operation in AR, and on days 30, and 90 in CR ($n = 5$, respectively). As a control, syngeneic lung transplantation was performed from BN to BN, and recipients were sacrificed on the same days as in AR ($n = 5$).

Specimens were quickly frozen, processed routinely, and studied histologically and immunohistologically. Subsets of infiltrating lymphocytes and macrophages were identified using a panel of antibodies. This panel included antibodies to TNF-α, IL-1β, IFN-γ, IL-4, OX6 (class II antigens), ED1 and ED2 (macrophages), W3/25 (CD4), and OX8 (CD8).

Results

Histologically, AR showed features of latent phase (day 1), vascular phase (day 3), and alveolar phase (day 5), according to Prop's classification [1]. In CR, mononuclear cells (MNCs) infiltrated around the vessels and bronchi, a feature reminiscent of the vascular phase of AR. Infiltrating MNCs were fewer at day 90 than at day 30, however, some large bronchi at day 90 showed protrusion of granulation tissue into their lumens.

Immunohistochemically, TNF-α was detected as early as day 1 in AR. Some MNCs around the venules were positive. The number of positive cells increased as the days progressed, and most MNCs showed positivity to TNF-α on day 5 (Fig. 1 a). In CR, some clusters of cells were positive around the bronchi and blood vessels (Fig. 1 b). On the sequential sections, bronchial epithelia adjacent to TNF-α positive cells were frequently positive for OX6 (data not shown).

IL-1β positive MNCs were detected around vessels on day 3 in AR, and increased in number on day 5. In CR, only scattered MNCs were positive for IL-1β.

IFN-γ positive cells were found on day 1 in AR, increased as the days progressed, and reached a maximum on day 5. Positive cells were observed in newly infiltrated areas, especially around venules (Fig. 2 a) or in the periphery of the lesions. Most MNCs were negative in CR (Fig. 2 b).

IL-4 showed similar patterns to those of IL-1β. A moderate number of MNCs were positive on day 5 in AR, but scant in CR. These data are summarized in Table 1.

Discussion

From the present study, the difference in cytokine production in acute and chronic rejection of rat lung allografts was clearly demonstrated. MNCs in AR produced

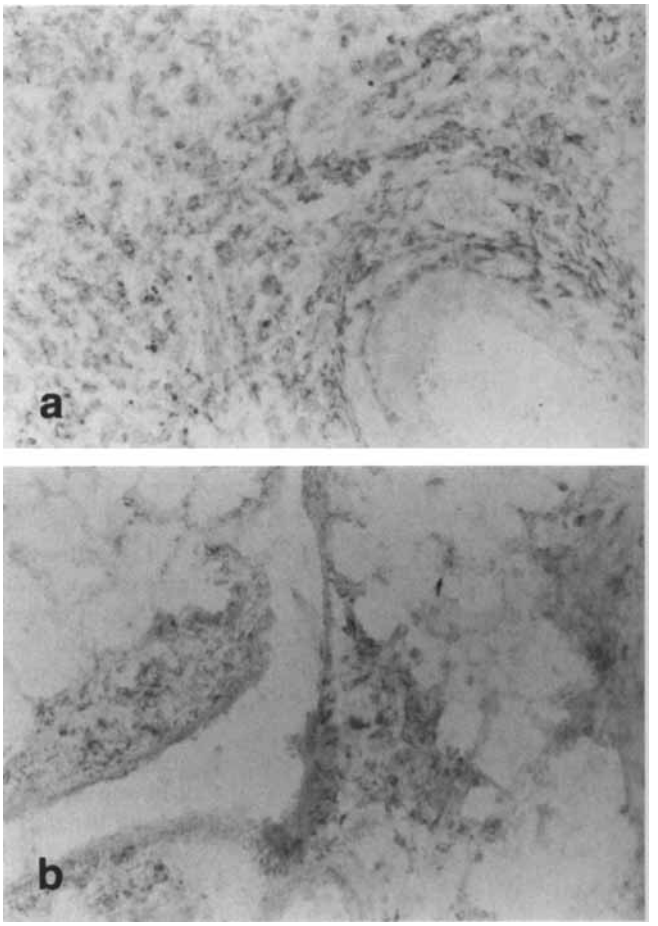

Fig. 1 a, b Reactivity for TNF-α is seen on most infiltrates in AR, day 5 (**a**), and on some cells in CR, day 30 (**b**)

every tested cytokine and increased in number as the rejection progressed; however, most MNCs in CR lacked production of IL-1β, IFN-γ, and IL-4. This difference seemed to play an important role in causing the different fates of the allografts, although their histologies are similar.

Recently, we demonstrated that the percentage of CD45RC positive cells in CD4-positive lymphocytes was significantly lower in CR than in AR [4]. CD45RC positive CD4-T cells were postulated as Th1 cells producing IFN-γ, whereas CD45RC negative CD4-T cells were postulated as Th2 cells producing IL-4 [5, 7]. Additionally, in some organs, activation of Th2 cells prevented the accelerated allograft rejection [8]. From these facts, we speculated the predominance of Th1 cells in AR and Th2 cells in CR which resulted in permanent acceptance. Both the considerable number of IFN-γ positive MNCs in AR and the insignificant number in CR were anticipated results, however, the lack of IL-4 positive cells in CR was unexpected. Although the studied cytokines were limited, a more intricate mechanism

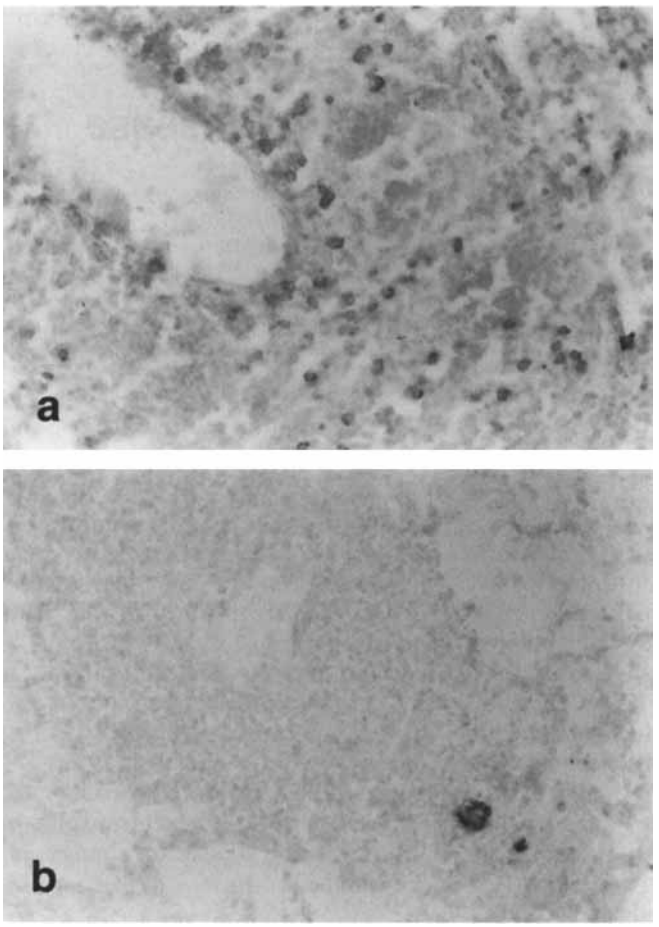

Fig. 2a,b Many infiltrating cells are positive for IFN-γ in AR, day 5 (**a**), while few cells are positive in CR, day 30 (**b**)

Table 1 Positive cells in the allografts (+/– Few, + some, ++ many, +++ most)

	Acute rejection			Chronic rejection
Day	1	3	5	30–90
TNF-α	+	++	+++	+
IL-1β	+/–	+	+	+/–
IFN-γ	+/–	+	++	+/–
IL-4	+/–	+	++	+/–

than Th2 predominance may be involved in preventing the rejection.

From the standpoint of chronic rejection, cellular production of TNF-α in CR is striking. Reactive cells were often gathered in the bronchial wall, and the bronchial epithelia adjacent to these positive infiltrates frequently expressed class II antigens. These facts may suggest that TNF-α upregulates class II antigens to produce chronic airway damage including bronchiolitis obliterans-like features observed in CR.

To summarize, cytokine production in acute and chronic rejection of rat lung allografts was quite different. Although cytokine production was limited in chronic rejection, Th2 predominance could not be demonstrated.

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