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Cytomegalovirus infection accelerates obliterative bronchiolitis of rat tracheal allografts

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Abstract A cascade of inflammation and injury of the airway wall followed by a fibroproliferative process that results in airway obstruction has been suggested as the explanation of the process of obliterative bronchiolitis (OB) in lung allograft recipients. To determine the impact of rat cytomegalovirus (RCMV) infection on the development of OB, heterotopic rat tracheal allografts were transplanted from DA donors to WF recipients immunosuppressed with 2 mg/kg per day cyclosporine A. Chronic RCMV infection was similarly established 8 weeks before transplantation in donors alone (D +/R –), recipients alone (D –/R +), and both donors and recipients (D +/R +). The control rats were left non-infected, but were similarly immunosuppressed. The results of this study demonstrate that both acute and chronic recipient RCMV infection, but not donor infection, amplify the development of experimental OB in the rat and suggest that RCMV infection-associated immune response, rather than the viral load in the graft, is essential for the development of the accelerated form of OB.

Key words Cytomegalovirus · Obliterative bronchiolitis · Lung · Trachea · Rat

Introduction

We have previously demonstrated that non-immunosuppressed tracheal allografts exchanged between major and minor histoincompatible rat strains develop histological changes similar to obliterative bronchiolitis (OB), and that these changes are primarily due to an alloimmune response to epithelium, associated with epithelial damage as well as marked proliferation of inflammatory cells and granulation tissue [1]. Our results further demonstrated that these changes can be inhibited by cyclosporine A (CsA) in a dose-dependent manner [1]. In this study, the impact of rat cytomegalovirus (RCMV) infection on the generation of experimental OB was investigated.

Materials and methods

Heterotopic rat tracheal allografts were transplanted from DA (AG-B4, $RT1^a$) donors into the omentum of WF (AG-B2, $RT1^v$) recipients and the recipients were given 2 mg/kg per day of CsA. For acute infection, recipients were inoculated intraperitoneally on the day of transplantation with 10^5 plaque-forming units of RCMV [2]. Chronic RCMV infection was similarly established 8 weeks before transplantation in donors alone, recipients alone, and both donors and recipients. The control rats were left non-infected, but were similarly immunosuppressed.

A segment of graft was fixed in 3% buffered paraformaldehyde, routinely processed, and embedded in paraffin. The grafts were examined histologically after sectioning and staining with Mayer's hematoxylin-eosin. To determine in vivo cell proliferation, the rats received bromodeoxyuridine by intravenous injection 3 h before sacrifice. Leukocyte subsets and the level of immune activation of inflammatory infiltrate were determined by the indirect immunoperoxidase technique using mouse monoclonal antibodies against rat determinants.

All data are expressed as mean ± SEM. The non-parametric Mann-Whitney U-test was chosen due to the small sample sizes

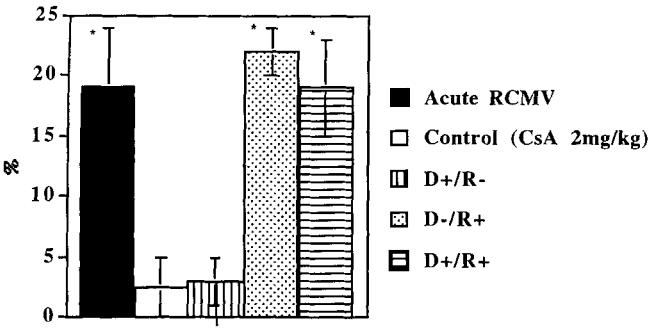

Fig. 1 Impact of acute and chronic RCMV infection in the development of luminal occlusion of tracheal allografts. (* $P < 0.05$, *CsA* cyclosporine A, *D +/R* – chronic RCMV infection in donors only; *D* –/R + chronic RCMV infection in recipients only, *D +/R* + chronic RCMV infection in both donors and recipients, RCMV rat cytomegalovirus)

and the inability to determine if the samples were normally distributed. The total variation between multiple groups was analyzed by the non-parametric Kruskal-Wallis one-way analysis by ranks, followed by the Dunn test for significances. *P* values less than 0.05 were regarded as statistically significant.

Results

RCMV infection significantly enhanced the generation of OB. Firstly, acute RCMV infection was linked to markedly enhanced MHC class II expression on the respiratory epithelium and prominent subepithelial inflammation of helper T cells (W3/25) and macrophages (ED1), with the prominence of lymphoid activation markers, MHC class II (OX 6), ICAM-1 (CD54), and IL-2R (CD25). Secondly acute infection induced a five-fold increase in luminal occlusion of the trachea, due to proliferating inflammatory and alpha-smooth muscle cell actin positive cells (Fig. 1). In chronic infection established in recipients alone, or both recipients and donors, the alterations were quite similar (Fig. 1). Chronic infection in donors alone significantly enhanced peritracheal inflammation, but showed no effect on subepithelial inflammation or luminal occlusion. Immunohistochemistry revealed that RCMV early and late antigen expression was quite similar in acute and chronic infection groups and that it occurred in the peritracheal area.

Discussion

Our results demonstrate that both acute RCMV infection and chronic recipient RCMV infection are significant risk factors for the development of enhanced obliterative changes in rat heterotopic tracheal allografts. Our findings suggest that RCMV replication in the tracheal allografts is unrelated to the development of pathological effects, and that a host immune response against RCMV is required for the enhancement of OB. This implies that either the viral load within the allograft is too low to evoke immune response, or the virus may escape immunocompetent T cells that recognize foreign peptides only in the context of host MHC I and II molecules. Studies determining the impact of RCMV on cytokine and growth factor ligand and receptor expression in the allograft are in progress.

References

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