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INADEQUATE ANTIBODY RESPONSE AGAINST RESPIRATORY VIRAL INFECTION IN LONG-SURVIVING RAT LUNG ALLOGRAFTS¹

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Lung transplant recipients suffer from a high number of viral infections. It has been suggested that the defense against viral infections is impaired in lung transplants. Therefore, we investigated in rat lung transplants whether antibody responses against an intrapulmonary viral infection were impaired in 3 groups of rats with: (1) BN-to-LEW allogeneic lung transplants, (2) LEW-to-LEW syngeneic lung transplants, and (3) nontransplanted LEW lungs. All rats (including those with nontransplanted, normal lungs) were treated with cyclosporine on days 2 and 3 after operation; this treatment is adequate to induce permanent graft acceptance of the allografts. Six months after transplantation, viral infections with Sendai virus (parainfluenza type I) were induced intratracheally. At day 0, immediately before infection, and at days 4, 7, 21, and 56 after infection, 4 rats in each group were killed for histological evaluation of the lungs. The number of antibody-positive cells in the bronchus-associated lymphoid tissue (BALT) in the lungs and in the spleen, and presence of the virus in the lungs were determined by immunohistology. Serum antibody titers were followed for 56 days after infection.

The allogeneically transplanted lungs failed to respond adequately against the virus: the number of antibody-positive cells in the BALT did not increase after infection, serum antibody titers were hardly detectable, and virus was present in the airways of the lungs up to day 21 after infection. In contrast, in the syngeneically and nontransplanted lungs, the number of antibody-forming cells in the BALT increased steeply until day 7, serum antibody titers rose until day 14, and virus could be detected only on day 4 after infection.

This study shows that in rat lung allografts, both the local antibody production in the BALT and the systemic antibody response against a respiratory viral infection are inadequate. As a consequence, the virus is present longer in these allografted lungs and can exert its damaging effect over a longer period of time.

These results may explain why lung transplants are so susceptible to viral infections.

Viral infections frequently affect the lungs of patients after heart-lung and lung transplantation and these infections are associated with high morbidity and mortality (1, 2). Lung transplant recipients suffer from infections twice as often as heart transplant recipients, despite comparable immunosuppressive protocols. So far, it is unclear why lung transplants are so susceptible to viral infections.

After a respiratory viral infection in the normal lung, antiviral antibodies are produced locally in the lung, in the bronchus-associated lymphoid tissue (BALT)* in animals such as rats and rabbits, and systemically in the spleen (3, 4). It is generally assumed that the local antibody production, especially the production of IgA in the BALT, is the most important first defense against an intrapulmonary viral infection (3-6). Whether the antiviral antibody production is affected by transplantation of the lungs is not known. However, in long-surviving rat lung allografts, we found the cell density in the BALT to be decreased, possibly as a result of rejection episodes (7). Similarly, in a clinical study, the number of immunoglobulin-positive cells in the submucosa of the transplanted lung was reduced in patients with chronic pulmonary rejection (8). This raises the question of whether in the transplanted lung a normal antibody response can be generated against viral infections.

In this study, therefore, we investigated the local and the systemic antibody responses and the clearance of virus from the airways after intrapulmonary infection with Sendai virus (parainfluenza type I) in long-surviving rat lung transplants with chronic rejection (9).

MATERIALS AND METHODS

Experimental design. Antiviral antibody responses and presence of virus in the airways were investigated in long-surviving lung transplants and in normal lungs after intrapulmonary infection with Sendai virus (parainfluenza type I). LEW rats were divided into 3 groups: in group 1, the rats received allogeneic BN lung transplants (n=20), in group 2, the rats received syngeneic LEW lung transplants (n=20), and in group 3, the rats received no lung transplants (n=20). All rats were immunosuppressed with a cyclosporine (CsA) injection on days 2 and 3 after transplantation. The nontransplanted rats of group 3 received the same immunosuppressive treatment. Six months later, 4 animals in each group were killed as noninfected controls (day 0). The remaining 16 rats of each group were infected

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* Abbreviation: BALT, bronchus-associated lymphoid tissue.

intratracheally with Sendai virus. At days 4, 7, 21, and 56 after infection, 4 infected animals in each group were killed for histological examination of the lungs and the spleens. Besides morphological evaluation of histopathology, the number of immunoglobulin-positive cells in the BALT and the spleen were determined semiquantitatively by immunohistology. Serum antibody titers were followed in the rats that survived until death at 56 days after infection. Clearance of the virus from the airways was determined by a monoclonal antibody staining against Sendai virus.

Rats. Young adult, male, specific pathogen-free LEW (RT1^l) and BN (RT1ⁿ) rats, weighing 250–350 g, were obtained from Zentral-Institut für Versuchstiere, Hannover, Germany. All animals received humane care in compliance with the Dutch regulations and law.

Lung transplantation. Left lung grafts were orthotopically transplanted in the thorax, according to the improved technique of Prop and Marck (10). Briefly, the donor lung was dissected and its vascular bed was flushed with cold saline. The recipient's left lung was removed and replaced with the donor lung; the pulmonary vein and artery were anastomosed first and then the bronchus.

To exclude technical failures, the transplanted lungs were monitored by chest roentgenography weekly during the first month and from then monthly until the day of infection with Sendai virus. All chest roentgenograms showed normal appearance of the transplanted lung at the day of infection.

All rats received CsA (provided by Sandoz Pharmaceuticals Corp., Basel, Switzerland), dissolved in olive oil, intramuscularly in a dosage of 25 mg/kg body weight on days 2 and 3 after lung transplantation. This treatment is adequate to induce permanent graft acceptance of the lung allografts. Normal rats also received CsA for 2 days.

Virus. In this study, Sendai virus (parainfluenza type I) was used to induce a respiratory infection. Culture and preparation of Sendai virus were performed by the ICLAS Reference Centre for Rodent Viruses (Department of Microbiology, University Hospital Nijmegen, Nijmegen, The Netherlands), as described previously (11).

Sendai virus was injected intratracheally, at a dose of 10³ plaque-forming units in 0.2 ml of medium. In a pilot study, this virus load induced mild pulmonary changes in normal LEW rats, with hyperplasia and lymphocytic infiltration of the bronchial epithelium and mild perivascular lymphocytic infiltration, which were transient.

Histology. For histological investigation of the lungs and spleens, the rats were exsanguinated under ether anesthesia. Heart and lungs were removed from the thoracic cavity and the spleen was taken from the abdomen. The lungs were intratracheally infused with optimum cutting temperature compound (Tissue-tek II; Lab-Tek Division, Miles Laboratories Inc., Naperville, IL) diluted 1:1 in PBS. Left and right lungs were separated at the hilar region and each lung was cut into 2 halves through the main bronchus to get longitudinal sections including the main bronchus. The spleen was cut in 2-mm-thick slices. One half lung and 3 slices of each spleen were embedded for paraffin sections. The other half lung and 3 slices of each spleen were snap-frozen in liquid nitrogen and stored at –80°C for immunohistological evaluation. Paraffin sections of lungs and spleens were stained for light microscopy with hematoxylin and eosin and methyl green pyronin.

Immunohistochemistry. The antibody-positive cells in the BALT of the infected lungs and in the spleens were detected with the mAbs MARM, MARG, and MARA, directed against IgM-, IgG-, and IgA-containing cells, respectively (gift of Dr. F. Kroese, Department of Histology and Cell Biology, University of Groningen, Groningen, The Netherlands). Virus-positive cells were stained with mAb MAB 834–3 directed against parainfluenza I virus (Chemicon International Inc., Temecula, CA). Therefore, serial cryostat sections were cut at 6 µm and air-dried for 30 min. The sections were rinsed in PBS and then incubated at room temperature for 1 hr with the appropriate monoclonal antibodies. After washing 3 times in PBS, sections were incubated for 30 min with horseradish-peroxidase-conjugated rabbit-antimouse Ig (DAKO, Denmark). Peroxidase was revealed by

staining with 3,3'-diaminobenzidine tetrachloride. Sections were lightly counterstained with hematoxylin. To assess nonspecific staining, control sections were incubated with PBS instead of monoclonal antibodies.

Assessment of cell numbers. The number of antibody-positive (IgA, IgG, and IgM) cells in sections of the BALT and spleen was counted using a grid micrometer with 100 squares at a magnification of ×400. The number of antibody-forming cells was counted in 6 randomly selected areas in each BALT and in 6 B cell areas in each spleen.

The number of virus-positive cells was scored in a semiquantitative way, recognizing 4 cell scores in which – = no cells, + = 1–10 positive cells, ++ = 10–20 positive cells, and +++ = more than 20 positive cells per field of view at a magnification of ×400.

Serum antibody titers. Immediately before infection (day 0) and at intervals after infection (4, 7, 14, 21, 28, 42, and 56 days) blood samples for antibody titer measurements were obtained by retro-orbital puncture. The titer of Sendai-specific antibodies was determined with an ELISA titer plate assay using the direct binding method. Alternate columns of the titer plate were coated with parainfluenza virus or bovine albumin as control. Serum was added at a 1:50 dilution and serially diluted. Then enzyme-labeled goat anti-rat Ig was used as detecting agent. Titers were calculated based on the 2 log transformation of the last dilution showing positive reaction.

Statistical analysis. Means and standard deviations of the number of immunoglobulin-positive cells in the BALT and in the spleen were calculated for the different groups on the different time points. Mean systemic antibody titers and standard deviations in the different groups were calculated on the basis of the 2 log titers of the individual titers. Number of antibody-forming cells, serum antibody titers, and the number of virus-positive cells in the lungs in the different experimental groups were compared with the Mann-Whitney rank sum test for unpaired values. The number of antibody-forming cells and the number of virus-positive cells in the left and right lungs were compared with the Wilcoxon signed rank test for paired values. A *P*-value of less than 0.05 was considered statistically significant. All statistical calculations were performed with the statistical software package StatviewII for the Apple Macintosh computer.

RESULTS

Local Antibody Response in the BALT

Morphological immune response in the BALT. In the non-infected rats (day 0), the BALT of the allogeneically transplanted lungs was cell poor and small compared with the BALT in the syngeneically transplanted and nontransplanted lungs. After infection, no morphological immune response developed in the BALT of the allogeneically transplanted lungs. Activated lymphocytes and plasma cells did not appear in the BALT during the observation period of 56 days after infection (Fig. 1A). As a consequence, the BALT in these allogeneically transplanted lungs did not increase in size. In the BALT of the syngeneically transplanted and normal lungs, a massive morphological response started on day 4 after infection with the appearance of lymphoblasts. At day 7 after infection, large numbers of pyroninophilic lymphoblasts were present throughout the BALT. From day 21 through day 56 after infection, plasma cells were present in high numbers in the BALT. During the strong morphological immune response, the size of the BALT increased considerably and remained enlarged until day 56 after infection (Fig. 1B).

Numbers of antibody-positive cells in the BALT. Assessment of antibody-positive cells showed that already before infection the total number of antibody-positive cells in the

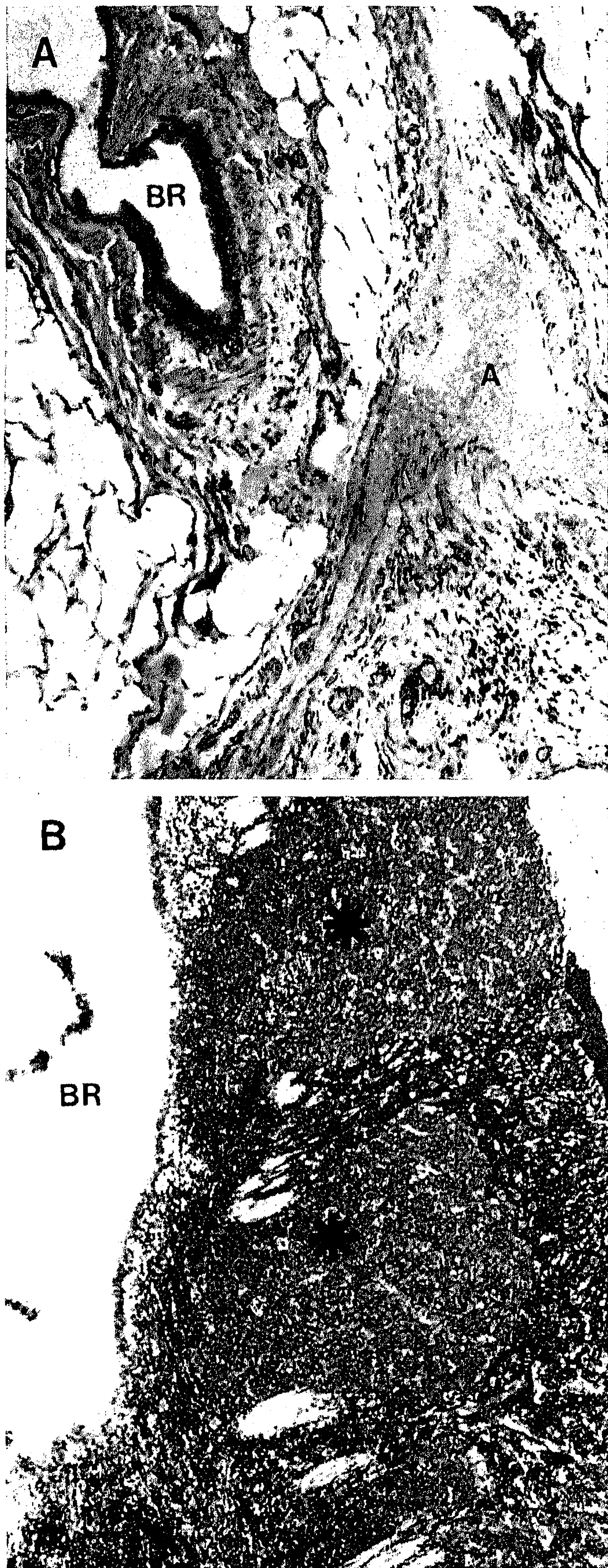


FIGURE 1. Morphological immune response in the BALT after respiratory viral infection in allogeneic lung transplants (A) and syngeneic lung transplants (B) on day 56 after infection (hematoxylin and

BALT of the allogeneically transplanted lung was significantly lower ($P < 0.05$) than in the syngeneically transplanted and nontransplanted lungs (Fig. 2).

After infection, the number of cells in the BALT positive for total Ig or IgA did not increase in allogeneically transplanted lungs (Fig. 2). In the syngeneically transplanted and nontransplanted lungs, the number of Ig-positive cells in the BALT started to increase already on day 4 after infection. In particular, the number of IgA-positive cells increased quickly in the syngeneically transplanted and nontransplanted lungs (Fig. 2). These numbers remained high up to 56 days after infection. The results of the syngeneically transplanted lungs were in striking parallel to the results of the nontransplanted lungs.

The contralateral right (nontransplanted) lungs in all groups showed an increase in antibody-positive cells in the BALT which was the same as that in the nontransplanted left lungs.

Systemic Antibody Response

Morphological immune response in the spleen. Light microscopy of the spleens in all 3 groups showed a typical immunoproliferative response in the B cell areas (germinal centers and corona) and in the T cell areas (PALS). In the allogeneically transplanted rats, the intensity of the systemic immune response was weaker than in the syngeneically transplanted and nontransplanted rats: in the allogeneically transplanted rats, the plasma cell reaction was weak and the T cell response involved only parts of the PALSes. This resulted in smaller germinal centers in the spleens of the allogeneically transplanted rats than in the other groups.

Numbers of antibody-positive cells in the spleen. The number of antibody-positive cells in the spleens of the allogeneically transplanted group did increase, but was lower than in the syngeneically transplanted and normal groups ($P < 0.05$ on days 7 and 21) (Fig. 3), which corresponds with the weaker germinal center reaction in the allogeneically transplanted group after viral infection. In contrast to the BALT, the number of IgA-forming cells in the spleen remained low in all groups.

Serum antibody titer. Serum antibody titers after infection in all groups corresponded to the number of antibody-forming cells in the spleen. The antibody titers were significantly lower in the allogeneically transplanted group than in the syngeneically transplanted and nontransplanted groups ($P < 0.01$ on from day 7 after infection) (Fig. 4).

Clearance of Virus-Positive Cells from Bronchial Epithelium

Virus-positive cells could clearly be detected in the bronchial epithelium on day 4 after infection in all infected lungs

eosin, original magnification $\times 60$). (A) In allogeneic lung transplants, after infection, no immune response developed in the remnants of the BALT of these lungs (EP = epithelium, A = bronchial artery). Activated lymphocytes and plasma cells did not appear in the BALT. As a consequence, the BALT did not increase in size. (B) In the BALT of syngeneic lung transplants, a massive immune response developed, with the appearance of follicles (indicated by asterisks) containing lymphocytes in different stages of activation and plasma cells (EP = epithelium). During this strong immune response, the size of the BALT increased considerably.

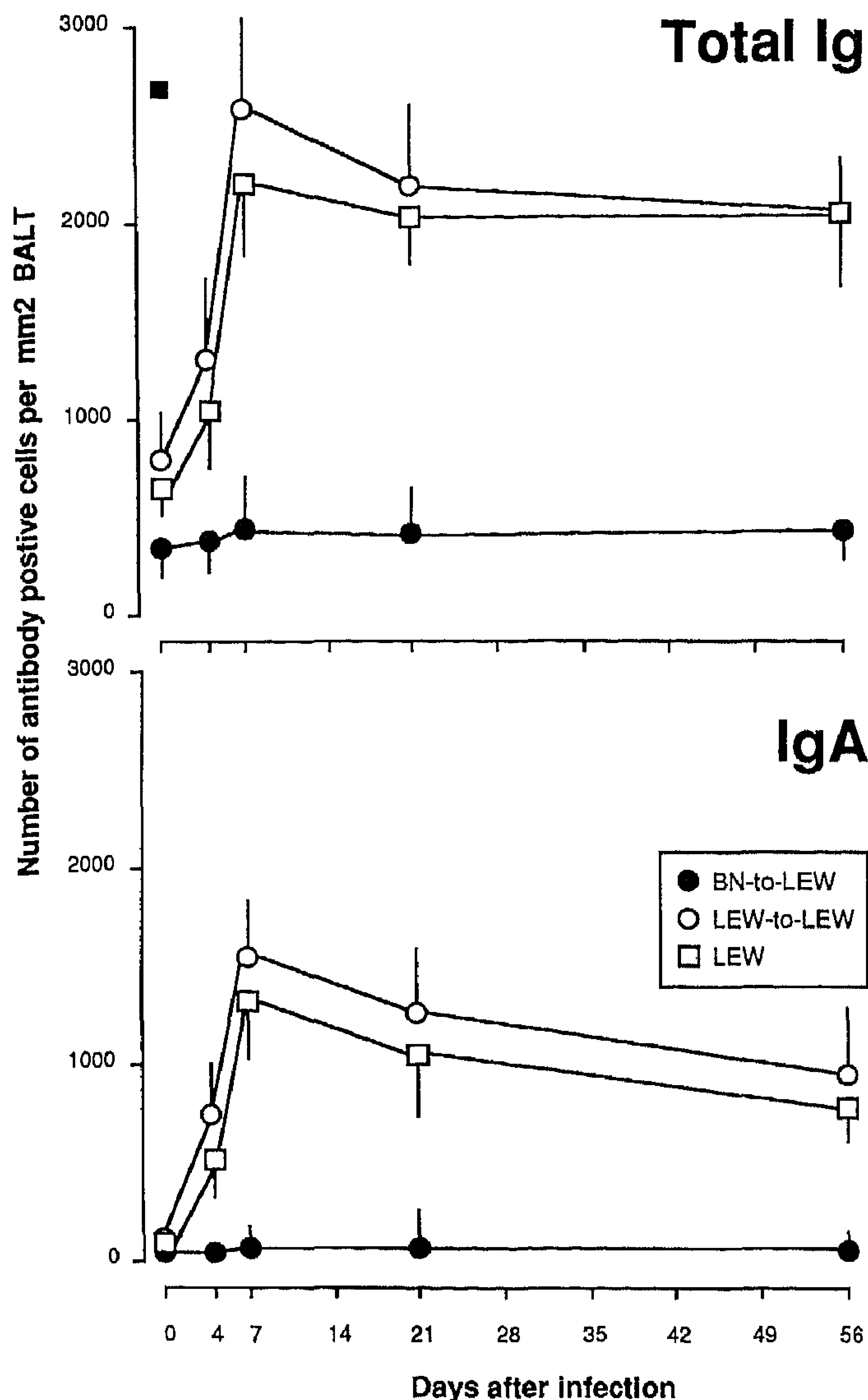


FIGURE 2. Number of antibody-positive cells in the BALT of the lung after respiratory viral infection. After infection, the number of cells in the BALT positive for total Ig or IgA did not increase in the allogeneically transplanted lungs (●). In the syngeneically transplanted lungs (○) and nontransplanted lungs (□), the number of Ig-positive cells in the BALT had already started to increase on day 4 after infection. In particular, the number of IgA-positive cells increased quickly in the BALT of the syngeneically and nontransplanted lungs. Ig and IgA cell numbers were significantly lower in the allogeneically transplanted lungs than in the syngeneically and nontransplanted lungs on all corresponding time points ($P < 0.01$).

(Fig. 5, A and C). The distribution of the virus was more widespread in the allogeneically transplanted lungs than in the syngeneically and nontransplanted lungs, affecting also the bronchioles (Table 1). Furthermore, clearance of the virus from the epithelium was slower in the allogeneically transplanted lungs than in the syngeneically transplanted and nontransplanted lungs (Table 1). In the allogeneically transplanted lungs, virus-positive cells were still present in the epithelium of the airways on day 21 after infection (Fig. 5B). In contrast, in the syngeneically transplanted and normal lungs, virus-positive cells could not be detected in the epithelium of the airways anymore after day 4 after infection (Fig. 5D).

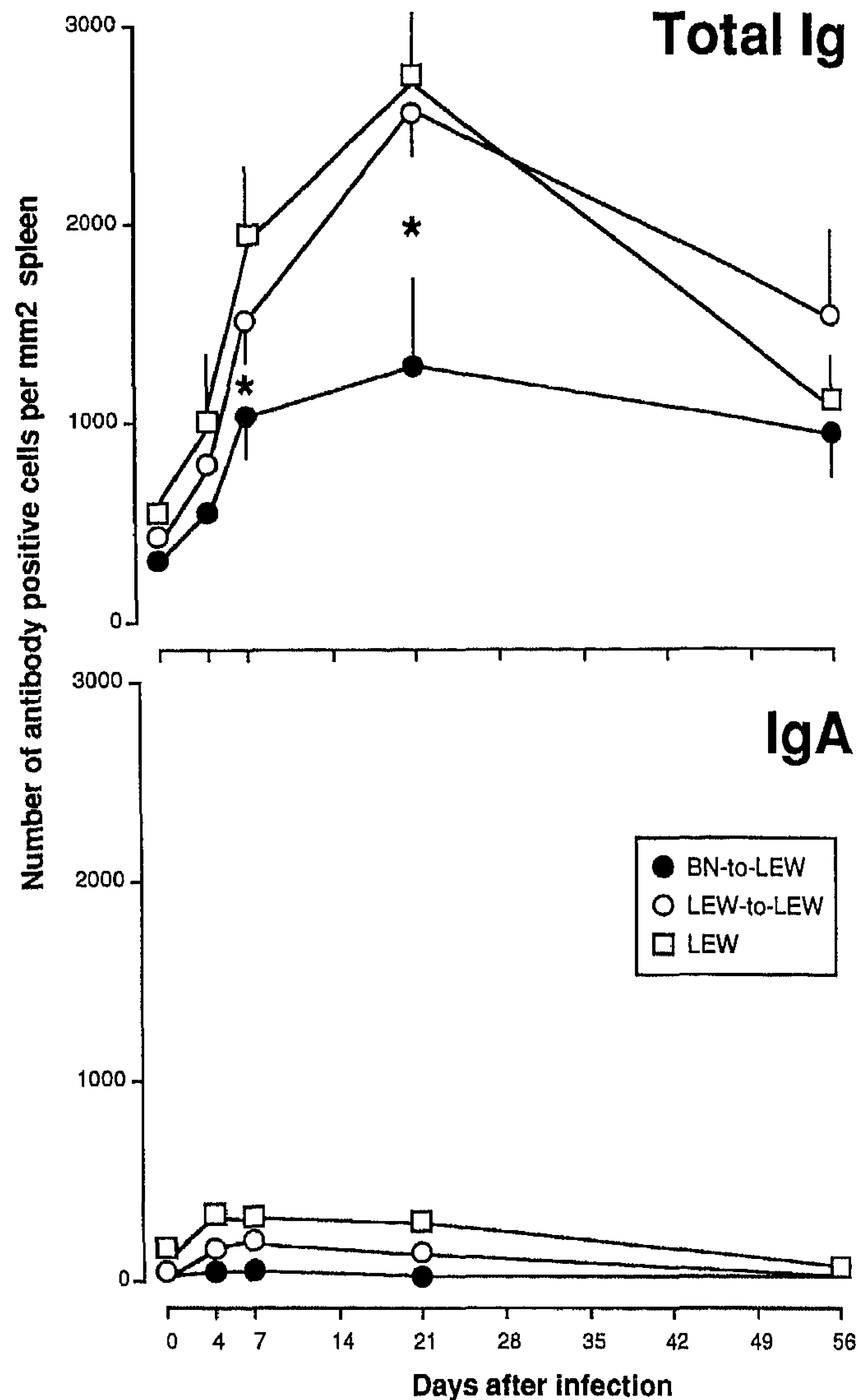


FIGURE 3. The number of antibody-positive cells in the spleen after respiratory viral infection. The number of total antibody-positive cells in the spleens of the allogeneically transplanted group (●) did increase, but was lower than in the syngeneically transplanted (○) and nontransplanted (□) groups ($*P < 0.05$ on days 7 and 21). Number of IgA-positive cells virtually did not increase in either of the 3 groups, without any difference among the groups.

lium of the airways anymore after day 4 after infection (Fig. 5D).

The contralateral right lungs in all groups showed a similar pattern of presence of the virus as the syngeneically transplanted and normal left lung; also, in these lungs, virus was only found on day 4 after infection.

DISCUSSION

This study demonstrates that in rats with allogeneic lung transplants, both the local antibody response in the BALT and the systemic antibody response against a respiratory viral infection are inadequate. This is supported by the finding that the virus is cleared more slowly from the lung allografts than from syngeneically transplanted lungs and nontransplanted lungs.

In normal lungs, an important component of the defense

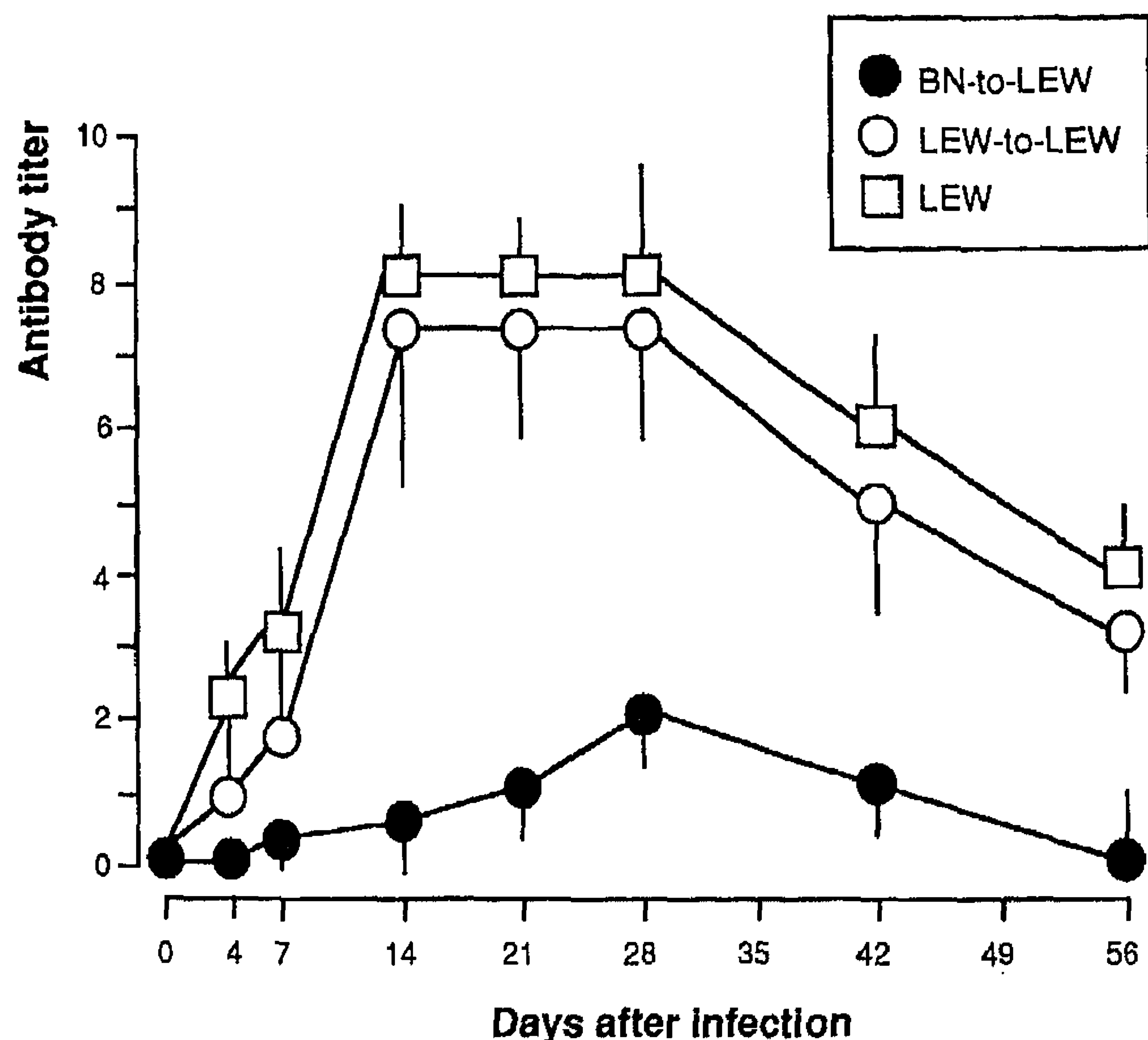


FIGURE 4. Serum antibody titer after respiratory viral infection detected with ELISA titer assay. The serum antibody titers were significantly lower in the allogeneically transplanted group (●) than in the syngeneically transplanted (○) and nontransplanted (□) groups ($P < 0.01$ from day 7 after infection).

system against respiratory viral infections has been attributed to antibody production locally in the BALT (3–5, 12). Local IgA production is particularly important in preventing infection by the virus of the bronchial epithelium by blocking of viral antigens (3–5). It has been shown that patients with an impaired IgA production are predisposed to pulmonary viral infections (5, 13). Our present study demonstrated that allogeneic rat lung transplants are not capable of generating an antibody response in the BALT. After infection with Sendai virus in the allogeneic lung transplants, a morphological immune response in the BALT did not occur and the number of IgA-containing cells in the BALT did not increase. A similar observation has been made in a study of the immune responses in rat small bowel transplants (14). In that study, no local IgA antibody response occurred in the Peyer's patches (the local lymphoid tissue in the small bowel) after immunization with cholera toxin. Taken together, these results suggest that the local lymphoid tissue, i.e., BALT in the lungs, does not function properly after allogeneic transplantation.

Explanations for the impaired function of BALT in lung allografts may be found in the structural changes of its lymphoid tissue. Already prior to infection, the surface area and cell density of the BALT (7) and the number of Ig-containing cells in the BALT (Fig. 2) are decreased in the allografts. Two causes have been suggested to be responsible for these structural changes, considering the fact that these changes occur only in the allogeneic and not in the syngeneic lung transplants. First, the BALT in the lung allografts may be damaged by rejection because of its high immunogenicity (15). Such damage by rejection was supported by findings in two clinical studies. In one report, a decrease in the number of Ig-containing cells in the submucosa of transplanted lungs was associated with chronic rejection (8). Another clinical study reported fibrosis of concomitantly transplanted hilar

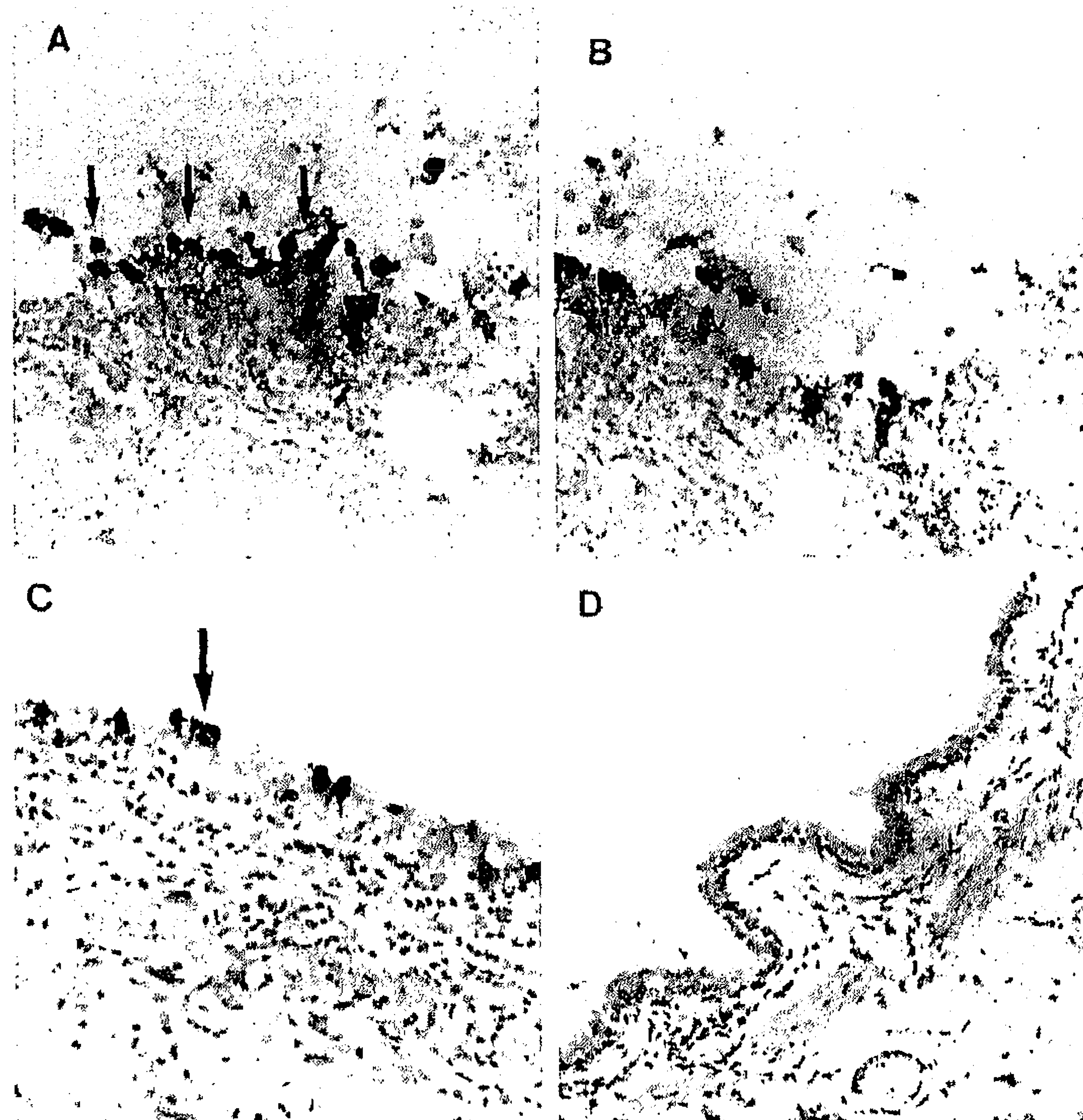


FIGURE 5. A–D. Presence of virus in the bronchial epithelium of allogeneic lung transplants (A and B) and syngeneic lung transplants (C and D) 4 days after infection with Sendai virus (A and C) and 21 days after infection (B and D). (Cryostat sections were stained with an mAb against Sendai virus; original magnification $\times 125$). (A) In allogeneic lung transplants on day 4 after infection, large numbers of virus-containing epithelial cells could be detected in the large airways (arrows). The epithelium is hyperplastic and necrotic. (B) On day 21 after infection, significant numbers of virus-containing cells could still be detected in the large airways. The epithelium is still hyperplastic. (C) In syngeneic lung transplants on day 4 after infection, virus-containing cells could clearly be detected in the epithelium of the large airways (arrows), but in lower number numbers than in the allogeneic lung transplants. The epithelium is hyperplastic, but not necrotic. (D) On day 21 after infection, virus could not be detected in the epithelium anymore. The epithelium is completely normal.

lymph nodes in heart-lung transplants with rejection episodes (16). Second, the BALT may be depleted of lymphocytes if migration of recipient lymphocytes to the BALT in the lung allografts is disturbed. To our knowledge, no studies have been published about migration of recipient lymphocytes into the BALT of allogeneic lung transplants. Results from a current animal study indicate that fibrosis of the BALT in long-surviving allogeneic rat lung transplants hampers normal migration of recipient lymphocytes indeed (17). It is quite conceivable that these structural changes prevent a proper function of BALT after respiratory viral infections.

Besides the local antibody response, the systemic antibody response in spleen and blood also was impaired in the allografted animals; the cause of it is unclear. One could imagine that induction of antiviral responses in the spleen is already affected in allografted rats. As mentioned above, the numbers of recipient lymphocytes migrating through the lung allograft, or at least through its BALT, are largely reduced. On top of that, the uptake of antigens from the airways into the BALT has been found to be blocked, proba-

TABLE 1. Virus-positive cells in the epithelium of the airways

	Days after infection				
	0	4	7	21	56
Allogeneically transplanted rats					
Left lungs					
Large airways	-	++	++	+	- ^a
Bronchioles	-	++	++	+/-	- ^a
Right lungs					
Large airways	-	+	-	-	-
Bronchioles	-	+/-	-	-	-
Syngeneically transplanted and nontransplanted rats					
Left lungs					
Large airways	-	+	-	-	-
Bronchioles	-	+/-	-	-	-
Right lungs					
Large airways	-	+	-	-	-
Bronchioles	-	+/-	-	-	-

^a Virus-positive cells were present significantly longer in the epithelium of the allogeneically transplanted lungs than in the airways of the syngeneically transplanted and nontransplanted lungs.

bly by a fibrotic layer isolating the BALT (17). In this way, a proper surveillance and peripheral sensitization of lymphocytes may be prevented. Absence of peripheral sensitization will prevent the induction of the systemic response against respiratory infections. One can also imagine that the systemic response is affected after its induction by a suppressive bystander effect: suppressor mechanisms of the alloresponses, involved in maintaining the graft, interfere with the antiviral responses. Bystander suppression of alloresponses and other immune responses has been shown in various experimental models (18). In parallel, we found an equal systemic suppression of antiviral responses when rats with long-surviving heart and spleen allografts (19) were infected by the intrapulmonary route with Sendai virus: the systemic antibody response was equally low as in the allografted group in this study (for 4 rats, the average peak titer was 3 ± 0.5 on day 21 after infection). Irrespective of the cause of the reduced systemic antibody response, it seems to have little influence on the clearance of the Sendai virus from the airways. This is demonstrated by our present finding that virus was cleared normally from the contralateral, right nontransplanted lung in the allogeneically transplanted animals. We attribute the normal clearance of the virus to the adequate antibody production in the BALT of these contralateral right lungs. These findings are an illustration of the idea that a proper function of the BALT is most essential for an adequate response against a respiratory viral infection.

Not all responses against intrapulmonary antigens are suppressed in lung allografts. In a previous study, we found that a normal serum antibody titer was generated after instillation of sheep RBC in long-surviving rat lung allografts (20). These antibodies, however, are not produced in the BALT of the donor lung, but in the paratracheal lymph nodes (21). Also, the induction of this response in the paratracheal lymph nodes is independent of the BALT, because these antigens are transported directly through lymph vessels to the lymph nodes (21). Whether T cell responses are normal in lung allografts cannot be excluded, because this was not investigated specifically in the present study. However, it is unlikely that they are normal, because morphological T cell responses were absent in the BALT and largely reduced in T

cell areas of the spleen. T cell responses are more important for defense against viral infections such as CMV (22) than against respiratory viral infections that depend heavily on IgA antibodies for primary defense.

A consequence of the impaired antibody responses against respiratory viral infections in the allogeneic lung transplants is that the virus is present in the airways over a prolonged period of time. During its prolonged presence in the airways, the virus can initiate an excessive inflammatory reaction with subsequent severe airway damage. In a previous study, this airway damage after viral infection in lung allogeneic lung transplants appeared to be very severe (23). We think that this severely damaging effect of viral infections in allogeneic lung transplants may contribute to the development of obliterative bronchiolitis, which is the major complication after clinical lung transplantation (24).

From this study, it is clear that the BALT in allogeneically transplanted lungs is not capable of generating an adequate antibody response against respiratory viral infections. As a consequence, the virus is present longer in these allografted lungs and can exert its damaging effect over a longer period of time. These results may explain why lung transplants are so susceptible to viral infections and why viral infections cause severe damage in these lung transplants.

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VARIATION IN THE LEVEL OF XENOANTIGEN EXPRESSION IN PORCINE ORGANS¹

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Hyperacute rejection of vascularized porcine to primate xenografts is initiated by the binding of xenoreactive natural antibodies to donor endothelium. We tested the hypothesis that the level of xenoantigen expression varies in the population of potential porcine donors and may determine the amount of binding of xenoreactive natural antibodies to a porcine organ perfused by xenogeneic blood. Two hundred ninety pigs were studied using an inhibition ELISA that quantitated the xenoantigen level on porcine plate-

lets. Based on this assay, the levels of xenoantigen expression in the population adhered to a normal distribution. Kidneys from pigs found to express high antigen levels and kidneys from pigs found to express low antigen levels were perfused with baboon blood using an extracorporeal circuit. In multiple experiments, a significant difference was observed in the amount of xenoreactive natural antibody adsorbed by high antigen versus low antigen organs. Normalizing for the weight of the perfused organs and for levels of natural antibody in individual baboons, high antigen organs adsorbed 3.6 ± 1.3 U of xenoreactive natural antibody/g and low antigen organs adsorbed -0.8 ± 1.0 U of xenoreactive natural antibody/g ($P < 0.002$). Immunopathology of tissues from the perfused organs demonstrated more deposition of IgM and C4 in high than in low xenoantigen organs. The quantitative relationship between binding of xenoreactive natural antibodies to platelets and to whole organs suggests that platelets are a valid representation of endothelial cell antigen expression in vivo. Despite the probable importance of Gal α (1-3)Gal as an epitope recognized by xenoreactive natural antibodies, differences in the binding to platelets or to organs of the GS-I-B₄ lectin that recognizes that sugar had no correlation with the

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