specific T cell clones from allergic patients, and by T cells in the bronchi of asthmatic subjects that are able to secrete cytokines, including IL5, with important effects on eosinophils and other cell types. There is also strong circumstantial evidence to support the role of the eosinophil as the main effector cell involved in the bronchial inflammation. The present study has shown that a large number of T cells, particularly of CD4+ subset, was recruited into the bronchi 7 days after the booster injection of antigen in actively sensitized guinea pigs. The other experimental groups, i.e., nonimmunized animals or sensitized only once, failed to show similar changes. Moreover, this increase in CD4+ cells was accompanied by an intense infiltration of eosinophils into the lung compartments studied. Despite the fact that CD8+ T cells were also increased in nonboosted, as compared to nonimmunized guinea pigs, they failed to show any significant changes in the boosted animals. These findings suggest that the lung inflammation following the booster injection of antigen is largely dependent on CD4+ T cells and eosinophils. Similar observations were made by Frew and coworkers. Using the isolated perfused lung preparation, it was previously demonstrated that the booster injection of antigen was responsible for the development of BHR to various agonists. It is therefore possible to speculate on an association between CD4+ T cells, eosinophils, and the emergence of BHR in this model, which opens new possibilities for the investigation of the immunopathogenesis of asthma and associated conditions and of their pharmacologic modulation.

ACKNOWLEDGMENTS: The authors are grateful to Drs. Schafer and Burger, from the Robert Koch Institute, Berlin, Germany, for the kind gift of the monoclonal antibodies H155 and H159, and to Mlle. Claude Ruffié and Mr. Jean Lefort for expert technical help.

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Changes in T Lymphocyte Subsets and Activation Following Chronic Antigen Inhalations in Monkeys*
Donald J. Souza; Robert H. Gundel, Ph.D.; Randy W. Barton, Ph.D.; Carol D. Stearns; Carol A. Torcellini; Eric J. Miner, B.S.; L. Gordon Letts, Ph.D.; and Craig D. Wegner, Ph.D.

Bronchoalveolar lavage (BAL) and blood lymphocytes were gated and analyzed by flow cytometry during the onset (day 0 to 10; antigen [Ag] on day 3, 5, 7) and sustaining (day 10 to 24; Ag on day 10, 12, 14, 17, 19, 21) of Ag-induced airway hyperresponsiveness as well as recovery (day 24 to 32) post-Ag in 11 adult male cynomolgus monkeys. Airway responsiveness was assessed by determining the concentration of nebulized and inhaled methacholine that induced a 100% increase in respiratory system resistance (PC20). The BAL CD4+ lymphocytes doubled by day 10 and tended to decline slightly thereafter (Table 1). Interestingly, CD8+ lymphocytes also tended to increase by day 10, reaching significance at day 24 and remaining elevated through day 32. The IL2 receptor positive (IL2R+) lymphocytes were undetectable at day 0, rose to 12% at day 10, and significantly decreased during the recovery period. Blood CD4+ cells did not change, while CD8+ cells were

<table>
<thead>
<tr>
<th>Day</th>
<th>Log. MCh PC20</th>
<th>Total Leukocytes, × 10⁴/mL BAL</th>
<th>% BAL Eos</th>
<th>% CD4+</th>
<th>% CD8+</th>
<th>% IL2R+</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.36±0.13</td>
<td>2.37±0.34</td>
<td>13.6±4.0</td>
<td>21.3±3.2</td>
<td>27.8±5.3</td>
<td>0.0±3.0</td>
</tr>
<tr>
<td>10</td>
<td>0.59±0.18†</td>
<td>7.68±1.30†</td>
<td>50.2±4.1†</td>
<td>41.1±2.9†</td>
<td>40.7±4.1</td>
<td>12.2±3.3†</td>
</tr>
<tr>
<td>24</td>
<td>0.61±0.22†</td>
<td>5.90±0.79†</td>
<td>52.0±4.7†</td>
<td>35.7±1.3†</td>
<td>53.8±2.5†</td>
<td>11.5±2.0†</td>
</tr>
<tr>
<td>27</td>
<td>0.90±0.20†</td>
<td>6.74±0.98†</td>
<td>32.8±5.8†</td>
<td>33.8±1.7†</td>
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</tr>
<tr>
<td>32</td>
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<td>31.4±3.8†</td>
<td>32.4±1.5†</td>
<td>57.6±3.2†</td>
<td>5.8±1.4†</td>
</tr>
</tbody>
</table>

*All values mean ± SE †p<0.05.

Acknowledgments: The authors are grateful to Drs. Brautigan and Burger, from the Robert Koch Institute, Berlin, Germany, for the kind gift of the monoclonal antibodies H155 and H159, and to Mlle. Claude Ruffié and Mr. Jean Lefort for expert technical help.

References
decreased at day 24 (51.11 ± 2.03 to 35.53 ± 2.37; p<0.01).

In summary, repeated Ag inhalation in primates induced significant changes in airway T cell subsets (CD4/CD8) and T cell activation (IL2R+). Furthermore, these changes (specifically T cell activation, IL2R+) appeared to parallel the pattern of airway eosinophilia (% BAL Eos) and hyperresponsiveness (PC20) from onset to recovery. These results provide a foundation for the evaluation of various anti-inflammatory/immune modulators as well as elucidating the roles of lymphocyte infiltration, proliferation, and activation in this model of Ag-induced airway inflammation and hyperresponsiveness.

Lung Allograft Rejection: Role of Tumor Necrosis Factor-Alpha and Interleukin-6*

Mark W Relfe, M.D.; Steven Kinkel, Ph.D.; Pamela Lincoln; Michael Deeb; Flavio Lupinetti; and Robert Strieter, M.D., F.C.C.P.

Lung transplantation has become a therapeutic option for a number of end-stage pulmonary disorders. Although the popularity of this procedure has exponentially increased over the last several years, lung transplant recipients have far more complications due to infections and allograft rejection as compared to patients receiving other solid organ transplants. While previous studies have established the relationship of allogeneic bronchus-associated lymphoid tissue and transplantation rejection, they have not demonstrated a relationship between cellular and molecular events of rejection. Recent studies have shown a significant role for both tumor necrosis factor (TNF) and interleukin-6 (IL6) in the immunoregulation of mononuclear cells, such as mononuclear phagocytes, and T and B lymphocytes. We postulated that these 2 cytokines may be instrumental in mediating lung allograft rejection.

To test this hypothesis, our laboratory used a well-established RT1 incompatible, specific pathogen-free, unmodified rat lung transplant model. Brown Norway (BN;RT1+) rat left lungs (allograft) were transplanted into Lewis (LEW;RT1+) rats, and LEW to LEW transplants represented syngeneic control samples. These animals were killed on days 1, 4, and 6 postimplantation. Full allograft rejection was reproducibly seen on day 6. Plasma anduffy-coat cells were isolated from anticoagulated blood. Bronchoalveolar lavage (BAL) fluid and lung homogenates were obtained from both the native and transplanted lungs. The TNF and IL6 bioactivity were measured by WEHI 164, subclone 13 and B9 hybridoma bioassays, respectively. Specificity for TNF or IL6 bioactivity in the above bioassays were determined by using specific neutralizing TNF and IL6 antibodies. The TNF and IL6 mRNA expression from lung homogenates oruffy-coat cells were determined by Northern blot analyses.

The TNF from lung allograft BAL was 347 ± 186 U/ml on day 1, declining to 18 ± 7 U/ml on day 4, and rising again on day 6 to 171 ± 103 U/ml, which was coincident with allograft rejection (n = 12). This represented a 5.9-, 1.5-, and 6.3-fold increase in TNF levels as compared to native lungs. Syngeneic lungs showed a similar pattern as the allograft lungs on days 1 through 4, however, TNF levels on day 6 were similar to the native lungs. The IL6 demonstrated a similar bimodal pattern as TNF from allograft transplanted lungs. The IL6 on day 1 was 3,400 ± 770 pg/ml, declining to 54 pg/ml on day 4, and rising again on day 6 coincident with lung rejection. 4,600 ± 420 pg/ml. This represented a 2.4-, 1-, and 10.5-fold increase in IL6 levels as compared to the native lung. The IL6 on day 6 from the syngeneic lungs was equivalent to values from the contralateral native lungs. The TNF and IL6 bioactivity from lung homogenates paralleled the levels from BAL, whereas lung allograft homogenate cytokine mRNA levels were maximally expressed on day 4. Plasma levels of TNF and IL6 were not significantly different from day 1 through 6, however, buffy-coat cell-derived steady-state TNF mRNA peaked on day 4 and remained elevated through day 6.

These data suggest that lung allograft rejection is associated with a local bimodal pattern of TNF and IL6 production. Whereas, TNF and IL6 were elevated from syngeneic transplants only on day 1. Furthermore, these findings suggest that elevated levels of TNF and IL6 on day 1 may reflect nonspecific inflammation due to ischemia-reperfusion of the lung transplant, while the elevated levels of these cytokines from the allograft on day 6 are associated with the full expression of lung allograft rejection.

Immediate Early Genes of Cytomegalovirus Antagonize the Inhibitory Effects of Cyclosporin A on Interleukin-2 Gene Transcription*

Lois J. Getts, M.D.; Martha Monick; Mark Stinski, Ph.D.; and Gary Hunninghake, M.D., F.C.C.P.

Cyclosporin A (CsA) is a useful immunosuppressive agent in patients with transplanted organs because it is a potent inhibitor of IL2 gene transcription. It is known that cytomegalovirus (CMV) infections trigger rejection of transplanted organs and that immediate early (IE) genes of CMV markedly upregulate transcription of the IL2 gene. These studies determined if the effects of CsA on IL2 transcription could be blocked by CMV IE gene products.

A plasmid with the promoter-regulatory region of IL2 upstream of the CAT gene (IL2CAT) was used to assess IL2 expression. The IL2CAT was cotransfected into Jurkat T cells with either a control IE plasmid or a plasmid with the bona fide IE1+2 genes downstream of the IE promoter. Without IE1+2 (control IE plasmid), CsA completely blocked the increase in CAT activity triggered by stimulating the cells with phytohemagglutinin and phorbol myristic

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