

## Rostrum

# Perivascular capillaries in the lung: An important but neglected vascular bed in immune reactions?

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In allergic and inflammatory immune reactions of the respiratory tract, leukocytes migrate into the different compartments of the lung. The air space can easily be sampled by means of bronchoalveolar lavage. However, the subset composition in the bronchial wall or the lung interstitium often differs considerably from that of the bronchoalveolar lavage fluid. A further compartment involved in very heterogeneous immune reactions in the lung has thus far not been mentioned: the periarterial space. In numerous experiments in different species with virus, bacteria, fungi, or allergens, there was not only a leukocyte infiltration of the bronchial lamina propria but also infiltration around branches of the pulmonary artery. This thus far neglected compartment consists of a different type of capillary. Thus it is important not to overlook this area in studies on allergic or inflammatory immune reactions of the lung. (*J Allergy Clin Immunol* 2002;110:209-14.)

**Key words:** Lung vasculature, compartments, periarterial capillaries, immune reactions

The lung is unique in its dual blood supply: the bronchial arteries are of relevance for oxygen delivery to the bronchial wall, including the bronchial lamina propria and epithelium, and the pulmonary arteries supply the alveolar area and the lung interstitium. The bronchial arteries, which show enormous species differences,<sup>1</sup> end in the microvasculature of the tracheobronchial tree, which has several ultrastructural unique features with major effects on permeability.<sup>1,2</sup> Structural and functional alterations in this vascular bed in allergic diseases (eg, asthma) have been described,<sup>3</sup> but only recently have these vessels been identified as potential targets for asthma treatment.<sup>4</sup> Leukocyte recruitment through this capillary bed shows some similarities to other organs with the sequential steps of rolling, adhesion, and transmigration.<sup>5</sup> However, the alveolar capillary bed supplied by the pulmonary arteries leading to the alveolar capillaries, with a diameter of only 5 to 6  $\mu\text{m}$ , do not allow rolling

### Abbreviations used

CCR3: C-C chemokine receptor 3

OVA: Ovalbumin

but require a squeezing of leukocytes between the endothelial cells.<sup>6</sup> Nevertheless, a leukocyte-endothelial cell interaction also seems to be of major relevance, as documented with intravital microscopy studies of the rabbit lung.<sup>7</sup>

Thus leukocytes can reach specific sites in the lung during immune reactions and inflammation through 2 routes that differ in the expression of adhesion molecules and the size of vessel into which leukocytes emigrate (ie, postcapillary venules in the bronchial system and capillaries in the pulmonary system).<sup>5,8</sup> Lymphocyte subsets, such as effector cells, accumulate preferentially in the compartments supported by the bronchial blood supply in contrast to the lung parenchyma, which is fed by the pulmonary vessels.<sup>9</sup> An often neglected aspect is that in the mouse no effective intrapulmonary bronchial arteries exist, as has been documented in morphologic<sup>10</sup> and, more recently, functional experiments.<sup>11</sup> Thus the prerequisite for the exit, the bronchial postcapillary venules, into the bronchial wall does not exist in this frequently used animal model.

In addition to these generally accepted entry sites for leukocytes into the lung, there is obviously a third pathway. In many different animal models of inflammation or allergic reactions, an extensive accumulation of leukocytes has been described in the perivascular space, an area around larger pulmonary arteries. Why is edema development most pronounced there, and how do the leukocytes get there? A unique vascular bed might be the reason for these often described but hardly discussed observations.

## A UNIQUE CAPILLARY BED SURROUNDING PULMONARY ARTERIES

Almost 20 years ago, Guntheroth and Lucht<sup>12</sup> examined latex casts of rat lungs using scanning electron microscopy. The airways were filled with glutaraldehyde, with a resulting transmural pressure of 10 cm H<sub>2</sub>O. The results were striking. In addition to the alveolar area

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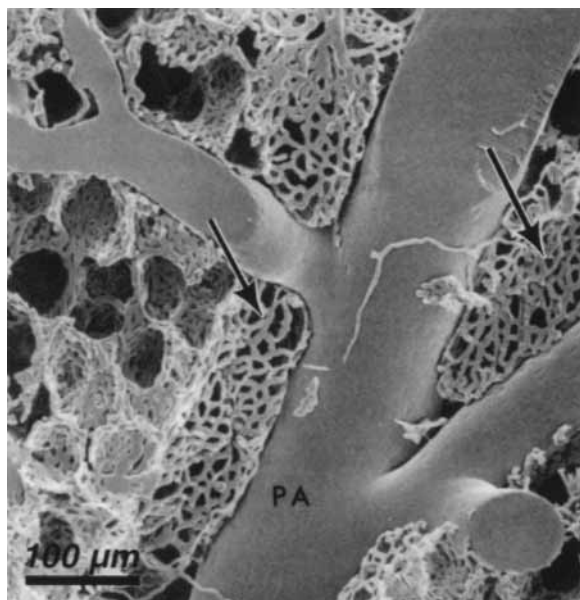


**FIG 1.** Scanning electron micrograph of a vascular cast of a rat lung showing a pulmonary artery (PA) with 2 branches and the unique periarterial capillaries (arrows) running more or less parallel to the artery, which show the typical honeycomb pattern and obviously differ from the alveolar capillaries. There seems to be a clear border between these 2 types of capillaries. Reproduced from Guntheroth and Luchtel,<sup>12</sup> with permission of the publishers.

with intersecting tubules, there was a different type, a thin subpleural layer, and even more relevant for this topic, long tubules of a relatively thin network of capillaries were found around pulmonary arteries (Fig 1). These observations were confirmed in a recently published study in adult Wistar rats using similar techniques.<sup>13</sup> The hypothesis is put forward that this perivascular capillary bed differs from the alveolar capillaries and is the reason for early edema formation and leukocyte infiltration in this area of the lung (Fig 2).

### PERIVASCULAR LEUKOCYTE INFILTRATION IN DIFFERENT MODELS OF LUNG INFLAMMATION

Despite an enormous variation in the microbial and other causative agents used to induce inflammatory or asthma-like reactions, a rather constant finding is perivascular leukocyte infiltrations in the lung (Fig 3). In some models inflammatory cell infiltration around intrapulmonary venules has also been described.<sup>14</sup> In the vast majority of figures published in animal models of allergic or inflammatory cell infiltration, branches of the pulmonary arteries are shown. Thus periarterial infiltrations are the predominant findings. Only selected examples of such studies will be quoted. These models include mice infected with respiratory syncytial virus,<sup>15</sup> bacteria (eg, *Streptococcus pyogenes*<sup>16</sup> or *Haemophilus influenzae*<sup>17</sup>), *Mycoplasma pneumoniae*,<sup>18</sup> *Mycobacterium tuberculo-*

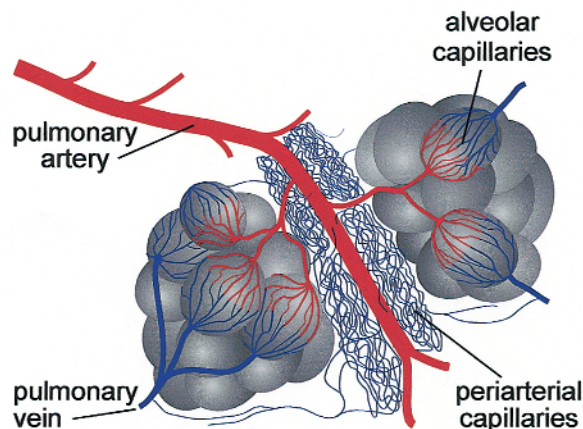


**FIG 2.** Schematic drawing demonstrating the basic pattern of the well-known alveolar capillary network and the different course and branching pattern of the capillaries surrounding the branch of the pulmonary artery.

*sis*,<sup>19</sup> or bacterial superantigen.<sup>20</sup> Often, asthma models have been performed in mice, such as with ovalbumin [OVA]<sup>21</sup> or *Aspergillus fumigatus* as an allergen,<sup>22</sup> or combined with treatment models, such as blocking the late phase of pulmonary inflammation after OVA exposure by means of the human platelet-activating factor acetylhydrolase.<sup>23</sup> Hamada et al<sup>24</sup> documented perivascular infiltration when they combined the allergen OVA with residual oil fly ash in young animals only. In other models used for studying the role of dendritic cells<sup>25</sup> or aerosol exposure after transfer of human tissues from sensitized patients<sup>26</sup> in severe combined immune deficient mice, a perivascular leukocyte infiltration was also obvious. The perivascular accumulation of lymphocytes is independent of TNF- $\alpha$  because in TNF- $\alpha$  knockout mice comparable infiltration has been noticed.<sup>27</sup> The role of adhesion molecule blockade, such as blocking of vascular leukocyte adhesion molecule 4 or E- and P-selectin in hypersensitivity pneumonitis,<sup>28,29</sup> was evident in preventing perivascular leukocyte accumulation. Obviously, it is a more general phenomenon and not restricted to inflammatory reactions to microbial antigens, which can cause these periarterial accumulations; an unspecific intratracheal challenge with sheep red blood cells also produced such a reaction in primed mice.<sup>30</sup> Other groups transferred alloreactive T<sub>H</sub>1 cells to the lung<sup>31</sup> or induced T<sub>H</sub>2-type foci around pulmonary blood vessels.<sup>32</sup> The cytokines produced by the T<sub>H</sub>2 lymphocytes IL-4 and IL-13 are critical for the induction of airway hypersensitivity but prevented peribronchial and perivascular leukocyte infiltrations in mice.<sup>33</sup> Surprising observations were documented in a recent article: when the transcription factor of T<sub>H</sub>1 lymphocytes (T-bet) was knocked out, there was a massive perivascular infiltration, with eosinophils and lymphocytes in the lung without previous antigen exposure.<sup>34</sup> The T-cell recruitment to the perivascular space is dependent on signals mediated through CD28, which is

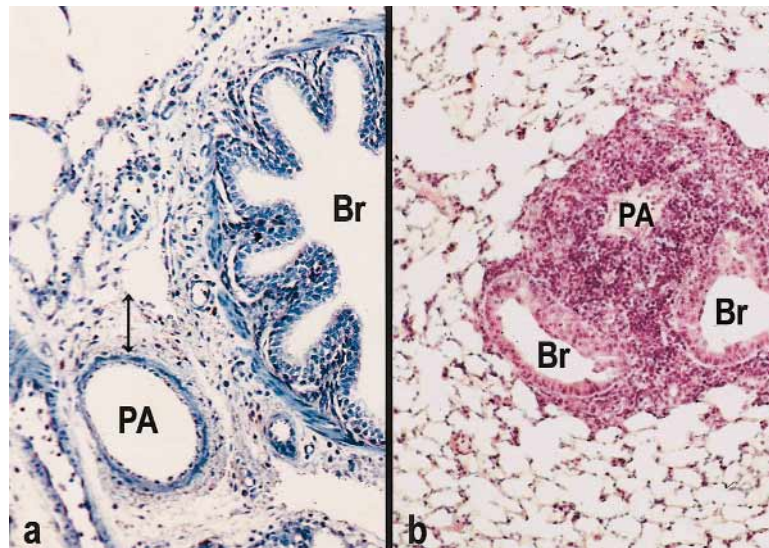


**FIG 3.** Histology of branches of the pulmonary artery (PA) and bronchus (Br). **a**, A pig lung after 30 minutes of normothermic isolated perfusion documenting edema formation (arrow) at this early time point (Giemsa staining; Pabst R and Binns R, unpublished data). **b**, An example of perivascular leukocyte infiltration in an MRL/lpr/lpr mouse, which is a model of autoimmune disease with lupus-type symptoms (Tschernig T and Neumann D, unpublished data).

relevant for the differentiation of antigen-specific T cells into  $T_H2$  cells.<sup>35</sup> Furthermore, chemokine receptors, such as C-C chemokine receptor 4, seem to play an important role in  $T_H2$  recruitment in serial antigen-exposure experiments.<sup>36</sup> C-C chemokine receptor 3 (CCR3) is another chemokine receptor described very recently.<sup>37</sup> In CCR3<sup>-/-</sup> mice there was a reduced eosinophil accumulation after allergen challenge around pulmonary arteries, with an obvious trapping in the vessel lumen and underneath the endothelial cells.<sup>38</sup> Thus CCR3 seems to be critical for transmigration of the basal lamina of these vessels. One study in particular is worth mentioning. In a genetic mouse model of immunodeficiency (aly/aly), there were no lymph nodes or Peyer's patches and a distorted spleen; however, a perivascular accumulation of lymphocytes in the lung was obvious, as was seen in control mice.<sup>39</sup> Because these perivascular infiltrations have been described not only in mice but also in rats and in the pig<sup>40</sup> and because the latter 2 species have bronchial arteries, this vascular compartment is obviously a more general phenomenon and can not be attributed to the lack of bronchial arteries in the mouse.

### DIFFERENTIAL ACCUMULATION IN THE PERIBRONCHIAL AND PERIVASCULAR SPACE

In most of the above-mentioned experiments, the authors focused on the peribronchial inflammation and mentioned the perivascular accumulations only in passing or in the legends to figures. In a few studies a more differential evaluation had been performed. Transgenic expression of GM-CSF and repeated aerosolized OVA

exposure resulted not only in eosinophilic but also lymphocytic (CD4 and CD8) infiltration preferentially around pulmonary arteries.<sup>41</sup> In mice sequentially infected with influenza followed by respiratory syncytial virus exposure, extensive lymphocytic and eosinophilic infiltrates were seen surrounding both pulmonary blood vessels and airways. However, an infection with either influenza or respiratory syncytial virus alone resulted in perivascular infiltration only, and no eosinophils were identified.<sup>15</sup> In the aly/aly mice (see above) there was no peribronchial infiltration, despite the massive perivascular leukocyte accumulation.<sup>39</sup> Different leukocytes contribute to these accumulations (eg, CD45R/B220<sup>+</sup> B lymphocytes along perivascular cuffs and CD4<sup>+</sup> T lymphocytes in contrast to a diffuse distribution of CD8<sup>+</sup> T lymphocytes in a tuberculosis model).<sup>19</sup> In a more recent study of the same group, it was documented that the majority of lymphocytes were perivascular and not within the parenchymal lesions.<sup>42</sup>

### HOW DO THE LEUKOCYTES REACH THIS PERIVASCULAR AREA?

On the one hand, it seems to be very unlikely that leukocytes would transverse the multilayered arterial wall, including the smooth muscles, and on the other hand, it has been shown, by using electron microscopic techniques, that leukocytes can use this route,<sup>43</sup> although this seems to be a very rare event. By using confocal laser microscopy on isolated perfused rat lungs, an upregulation of the intercellular adhesion molecule 1 in lung capillaries was documented without changes in arteriolar intercellular adhesion molecule 1 expression after

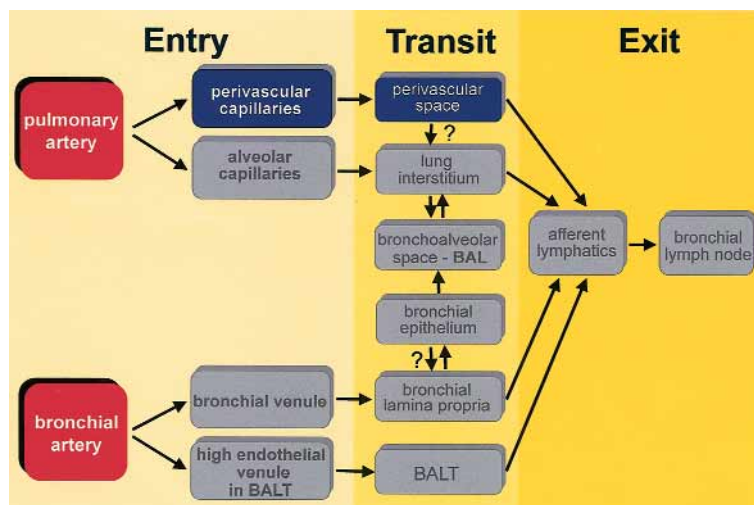


**FIG 4.** Schematic drawing of the compartments of the lung and the different blood supply and entry sites for leukocytes, their potential routes of transit through the parenchyma, and the exit to bronchial lymph nodes. The thus far neglected perivascular capillaries and perivascular space are shown at the top. *BALT*, Bronchus-associated lymphoid tissue.

**TABLE I.** Topics of future research on periarterial capillaries in the lung

|                                                                                                                                                                |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| What is the structural basis for the leakiness of the periarterial capillaries in the lung (eg, basement membrane differences)?                                |
| Are there species differences in the structure and function?                                                                                                   |
| What are the regulatory factors for leukocyte extravasation (eg, adhesion molecules, chemokines, and their receptors)?                                         |
| Is there preferential emigration of leukocyte and lymphocyte subsets?                                                                                          |
| Which cytokines regulate the microenvironment in the perivascular space, influencing apoptosis and local cell proliferation and resolving these accumulations? |
| Could these perivascular leukocyte accumulations be therapeutically prevented and thus influence inflammatory and allergic reactions in the lung?              |

bleomycin administration.<sup>44</sup> A further problem is to figure out how the signal reaches the endothelium of the capillaries. For example, after an intratracheal instillation of bleomycin, by 2 days later the mRNA of the tissue inhibitor of metalloproteases increases in the perivascular space, followed later by perivascular infiltration of leukocytes.<sup>45</sup> Macrophages accumulated around pulmonary arteries, and dendritic cells were near to bronchioles 24 hours after intratracheal instillation of the reagent in a delayed-type hypersensitivity reaction.<sup>46</sup> During the induction of inflammation, there was an increase in substance P-immunoreactive nerve endings within the perivascular cell infiltrates,<sup>47</sup> which might indicate a regulatory role of nerves in this phenomenon.

### EARLY EDEMA FORMATION IN THE PERIVASCULAR SPACE

A different aspect underlines the clinical relevance of the perivascular space of the lung in different species. In very diverse pathologic states, such as high-altitude pulmonary edema, hypervolemia, and alloxan toxicity in dogs, fluid accumulated in the perivascular space earlier than in the alveolar septa.<sup>48</sup> In highly controlled perfusion systems of the lungs of mice<sup>49</sup> or rats after endotox-

in exposure,<sup>50</sup> perivascular edema was frequently seen around large and medium arteries and veins. Similar observations (Fig 3) were made in isolated perfused pig lungs (Pabst R and Binns R, unpublished data) or at 3 hours after exposure of pigs to nebulized residual oil flying ash (W. Cascio, personal communication). Exposure of mice to LPS as an aerosol for 4 hours resulted in dramatic edema around the branches of the pulmonary artery.<sup>51</sup> Thus in various species this perivascular bed seems to be more inclined to leak under more diverse circumstances than the alveolar capillaries.

### CONCLUSION

The space around the branches of pulmonary arteries in the lung has been largely neglected, despite the early occurrence of edema formation and the accumulation of leukocytes in a wide variety of inflammation and allergy models in different species, which has thus far been mentioned only in passing. The perivascular capillary bed is proposed as a unique compartment, with specialized capillaries enabling a rapid exit of leukocytes (Fig 4). Many questions are still unanswered, and more experiments have to be performed (Table I). What is the function or pathophysiologic role of the accumulation of these cells

in this area? Is this capillary bed found in all species? Are there ultrastructural differences compared with other lung capillaries to explain the edema formation or leukocyte extravasation? Are there specific adhesion molecules on the endothelial cells of these capillaries? Could the exit be blocked, for example, by antibodies against adhesion molecules?

At present, there are many more questions than answers. In the future, the perivascular accumulations of leukocytes should no longer only be mentioned in passing or only documented in figures of allergy models and not in the text, which has been the case thus far because the peribronchial space has been the main feature of interest.

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