

Detection of CD4⁺ and CD8⁺ T-Lymphocytes in Lung Tissue of A/J-Mice by Immunohistology

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Introduction

CD4⁺ T-lymphocytes (T-helper/inducer cells) and CD8⁺ T-lymphocytes (T-suppressor/cytotoxic cells) are regarded as important cells in the pathogenesis of cigarette smoke-induced lung diseases (COPD, emphysema, asthma) in humans (Saetta et al., 1998; Takubo et al., 2002; Guerassimov et al., 2004). To allow investigation of a possible shift in the relative numbers of CD4⁺ and CD8⁺ T-lymphocytes after cigarette smoke inhalation, as seen in the human situation, we adapted immunohistological procedures as documented in the literature to our needs and established a method for staining the different T-lymphocyte subpopulations in lungs of A/J-mice thus enabling quantification of CD4⁺/CD8⁺ T-lymphocytes.

Methods

Animals/Tissue

The lung and the spleen of untreated male A/J-mice, bred under specific pathogen-free conditions (The Jackson Laboratory, Bar Harbor, Maine, U.S.A.) were taken for the establishment of the immunohistological staining protocol. The spleen was used as positive control where T-lymphocytes are located in the periarteriolar lymphoid sheath (PALS) of the white pulp.

Fixation and Slide Processing

The tissue for immunohistological investigation was fixed immediately after dissection with HOPE (Hepes Glutamic Acid Buffer Mediated Organic Solvent Protection Effect) (DCS Innovative Diagnostic System, Hamburg, Germany) solution according to a standard protocol. The lung was instilled via the trachea with the fixative under constant pressure of 20 cm H₂O. After paraffin-embedding, histological slides of 4 µm thickness were prepared.

Staining Procedure

Rat monoclonal antibodies were used for identification of CD4⁺ (Rt anti-Ms, Clone YTS191.1, Serotec) and CD8⁺ (Rt anti-Ms, Clone KT15, Serotec) lymphocytes. Monoclonal antibody binding was detected with the ABC-kit for peroxidase reaction (PK-6102, Vektor) and Diaminobenzidin (Dako) substrate.

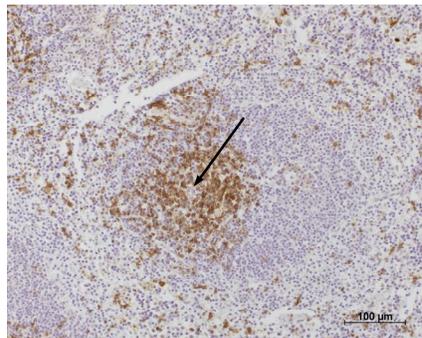
Discussion

For establishment of an immunohistological detection system of T-cell subpopulations, monoclonal antibodies against the CD4 and CD8 antigens were used. Using spleen tissue as the positive control, a specific staining of T-lymphocytes with both antibodies can be shown. The staining results from the lung tissue might allow the quantification of each cell population. The technique of immunohistological characterization of CD4⁺ and CD8⁺ lymphocytes in lung tissue might be a useful method to investigate the influence of T-lymphocytes during the development of lung diseases after inhalation of cigarette smoke in A/J-mice.

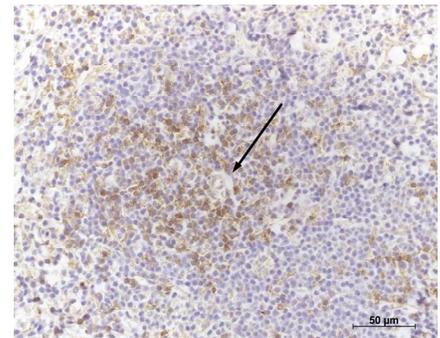
Results

Spleen: An intensive cell specific brown to yellow cytoplasmic staining of lymphoid cells mainly in the PALS of the white pulp can be seen after incubation with CD4 and CD8 antibodies (Pictures 1 and 2). Few scattered lymphoid cells in the red pulp and in the follicular center react positive.

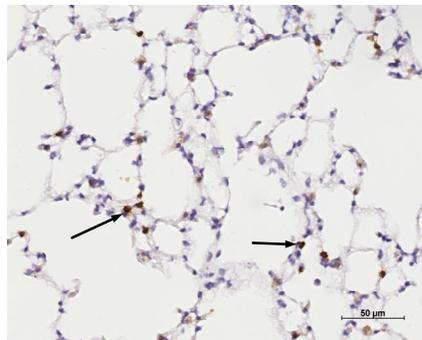
Lung: In the lung tissue positive stained cells were mainly located in the alveolar walls. The staining result after incubation with CD8 was slightly more intensive than after incubation with the CD4 antibody (Pictures 3 and 4).



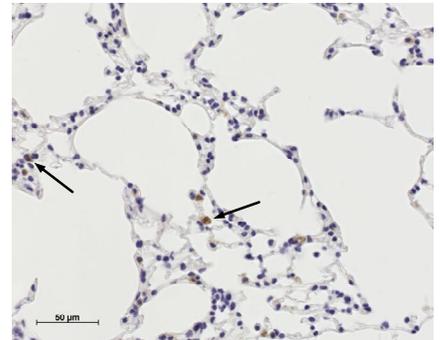
Picture 1: Spleen, mice, anti CD8-antibody, stained lymphoid cells are mainly located at the PALS (arrow), ABC-complex, peroxidase reaction, Diaminobenzidin, 200x



Picture 2: Spleen, mice, anti CD4-antibody, stained lymphoid cells are mainly located at the PALS (arrow), ABC-complex, peroxidase reaction, Diaminobenzidin, 400x



Picture 3: Lung, mice, anti CD8-antibody, stained cells are located inside the alveolar wall (arrow), ABC-complex, peroxidase reaction, Diaminobenzidin, 400x



Picture 4: Lung, mice, anti CD4-antibody, stained cells are located inside the alveolar wall (arrow). The amount of positive cells is slightly lower compared with the results after incubation with CD8 antibody, ABC-complex, peroxidase reaction, Diaminobenzidin, 400x

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