

Inflammatory Lung Diseases

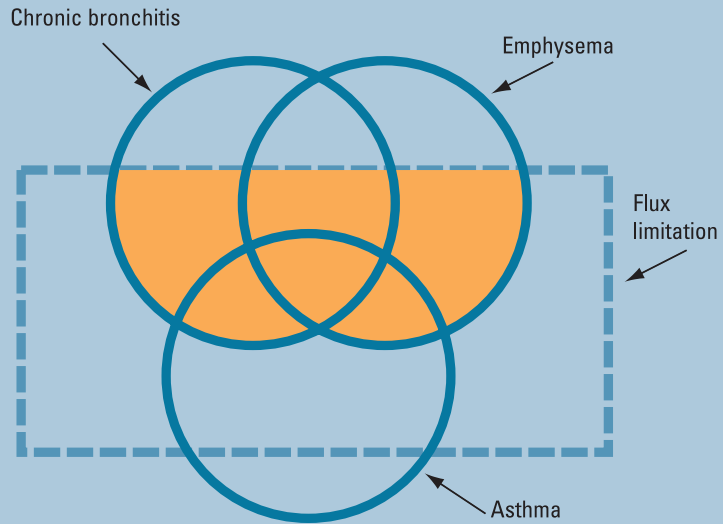
Chronic bronchitis and other respiratory diseases may be caused by inhaled particles. The Clinical Cooperation Group “Inflammatory Lung Diseases” of the GSF analyzes the mechanisms of the pathogenesis in cooperation with the Asklepios-Fachkliniken. With this work the Group opens up new avenues for the diagnosis and therapy of inflammatory lung diseases.

In Gauting near Munich the GSF has established the Clinical Cooperation Group (KKG) “Inflammatory Lung Diseases” on the campus of the Asklepios-Fachkliniken. This KKG was the first of its kind to transfer insight gained at the laboratory to clinical practice and vice versa to include clinical results in its further fundamen-

tal research. It is affiliated to the GSF Institute of Inhalation Biology and closely cooperates with the doctors of Fachkliniken (specialized hospitals) for pulmonology and thoracic surgery. This means that the scientists can, for example, obtain samples from lung patients of the hospital.

The Gauting KKG "Inflammatory Lung Diseases" focuses on environmental chronic obstructive pulmonary disease (COPD). There are overlaps with other clinical pictures, such as chronic bronchitis, asthma and emphysema. The orange areas are assigned to COPD.

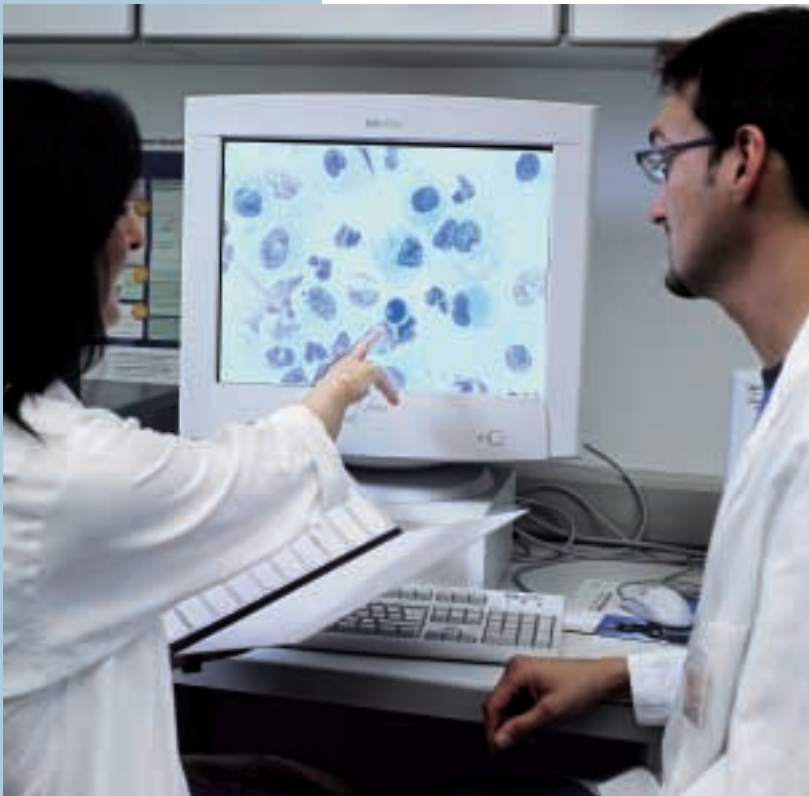
Chronic obstructive pulmonary disease = COPD



Fatal Consequences of Fine Particles

The KKG focuses on environmental chronic obstructive pulmonary disease (COPD). This includes chronic obstructive bronchitis and pulmonary emphysema. In emphysema alve-

olar tissue is destroyed, which results in a reduction of the internal surface of the lungs, which is important for gas exchange. COPD is one of the most frequent diseases worldwide and was the fourth most common cause of death in Germany in 2001. What makes this disease so fatal is that current therapies only mitigate the symptoms, they cannot halt the course of the disease altogether. COPD is also difficult to diagnose. Most patients with COPD show all three symptoms: chronic bronchitis, emphysema and excessive secretion of mucus.



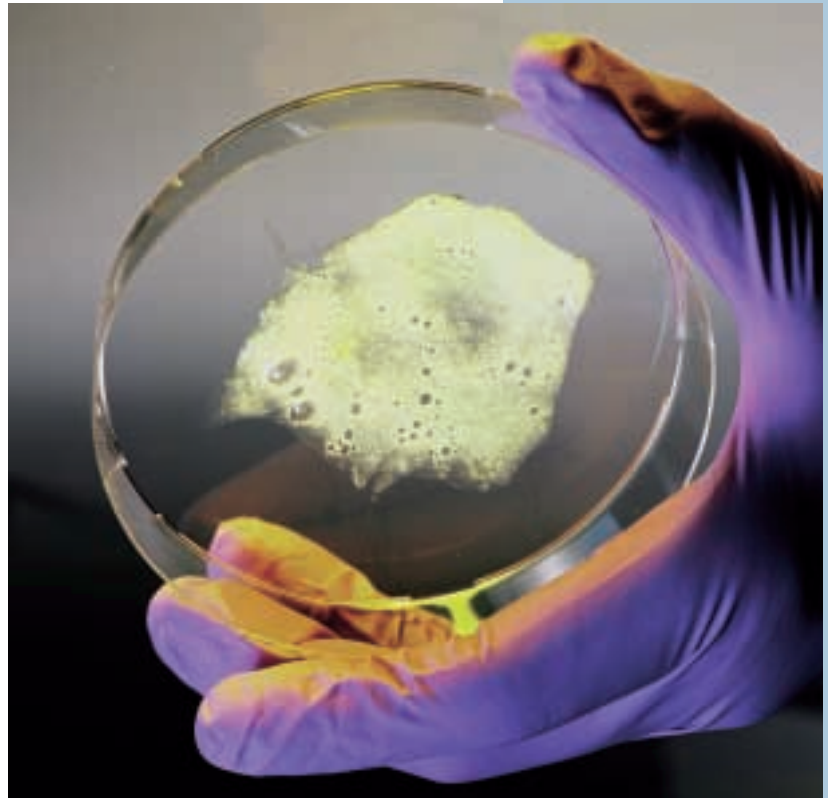
In the bronchial secretion of COPD patients the Clinical Cooperation Group showed a macrophage population of cells which are smaller than the macrophages known and are, therefore, called small sputum macrophages. The portion of this population is normally only approx. ten per cent of all macrophages, but may rise to up to 90 per cent in COPD patients. The cells seen on the screen are isolated from the sputum samples and applied to a slide. The cellular structures can be made visible by Papanheim staining, so that a morphological differentiation is possible.

Small Sputum Macrophages refer to COPD

The KKG investigates the influence of particles on the mechanisms of the development of COPD and wants to develop new diagnostic and therapeutic methods. For this purpose Dr. Marion Frankenberger, head of the Cooperation Group, and her team analyzed lung cells of COPD patients and discovered something very interesting: in the sputum of COPD patients, the scientists identified a new macrophage population with cells smaller in size than the known macrophages. Therefore, they were called small sputum macrophages. In healthy organisms macrophages are the main population of all white blood cells in this compartment. Their portion in the samples from the patients is quite different: their population is only 15 per cent, approx. 80 per cent of the cells are neutrophilic granulocytes. The portion of the small sputum macrophages, which normally account for only approx. ten per cent of all macrophages, can rise to up to 90 per cent in COPD patients. "This is also a way to distinguish asthma from COPD, since the concentration of the small sputum macrophages is only slightly elevated in asthma patients," Marion Frankenberger explains.

Inflammatory Genes Activated

Macrophages are of central significance in the respiratory tract and in the periphery of the lungs, the alveoli: they take up exogenic bacteria, viruses and also aerosol particles. The small sputum macrophages, which are found in greater quantities in COPD patients, produce large quantities of the tumor necrosis factor (TNF). This cytokine stimulates inflammatory reactions and thus contributes to the development of chronic obstructive bronchitis and helps sustain it. "We suspected that air-borne particles activate particular genes of these macrophages," says Frankenberger. The analysis of the gene expression confirmed this assumption: diesel exhaust particles and carbon particles have the effect that in the macrophage-like cell line (Mono Mac 6) higher amounts of the gene COX-2 are



expressed. Cyclooxygenase-2, which is the full name of the enzyme which is synthesized according to the design of the COX-2 gene, is involved in the oxidation processes in the lungs. If many COX-2 enzymes are active, a large number of oxidatively reactive substances is produced, which initially intensify the inflammatory reaction in the lungs. Following this, further messenger substances, such as leukotriene B₄ (LTB₄) and prostaglandin E₂ (PGE₂) are activated, which also influence the inflammation. LTB₄ has a stimulating effect, while PGE₂ contributes more to bringing the inflammatory process to a halt. Whether this actually works depends on the distribution of these biological signal substances and their receiver molecules, the receptors. "In an inflammatory reaction there is an interaction of many different steps," says Frankenberger. "We do not know yet what the exact structure of this network is. It is, however, certain that at some point in this chain the cytokine TNF is also activated, which in turn maintains the inflammation status in the cell." So in the investigated cell line Mono Mac 6 ultrafine particles support the gene expression of pro-inflammatory and anti-inflammatory mediators of the lungs.

Samples from induced sputum allow the non-invasive isolation of cellular material from the lungs. The results can give evidence of the inflammation status in the lungs. A new method applied in the KKG, the analysis of exhaled air – air flowing out of the lungs during exhalation – causes the patients even less discomfort.

In the search for effective therapeutic approaches for COPD Marion Frankenberger and her Clinical Cooperation Group have great hopes in vitamin A. It has a direct influence on the enzymes of macrophages and can thus halt damage to the lung tissue.



Whether the small particles also activate genes in cells of COPD patients and in healthy test subjects is an issue Frankenberger's group wants to investigate in the future. At some point the scientists hope to be able to use this knowledge to shut down inflammatory genes or to intensify anti-inflammatory processes.

Vitamin A against Tissue Degradation

To follow the course of inflammatory diseases in the lungs, doctors and scientists have to control inflammation markers regularly. Invasive methods and the taking of sputum have always caused patients with highly advanced COPD great discomfort. But this is now over: the Cooperation Group in Gauting uses markers in the exhaled air – the air flowing out of the lungs during exhalation – to control whether, for example, the patient responds to a cortisone therapy. If cytokines and lipid mediators, such as LTB_4 and PGE_2 , decrease during the therapy, the inflammation of the lungs has also receded.

Using markers in the exhaled air scientists from the KKG also want to check in the future whether vitamin A can stop tissue damage causing emphysema in the alveoli. First the scientists transferred vitamin A packed in little fat droplets, the liposomes, specifically to the macrophages in cell culture tests. Their

enzymes are known to decompose lung tissue. In healthy organisms the protease MMP9 (matrix metalloproteinase 9) and its inhibitor TIMP1 (tissue inhibitor of matrix metalloproteinase) are in the macrophages in equal concentrations. This ensures that exactly as much lung tissue is decomposed as new tissue is produced. In COPD patients, however, there is an imbalance: the decomposing enzyme MMP9 is present in much higher concentrations than its antagonist TIMP1. "It is clear that in this case the lungs continue to be digested," says Frankenberger. In this process vitamin A could come to the rescue: it reduces the protease MMP9 in cell lines, while it also activates the inhibitor TIMP1. So vitamin A supports the protection of the tissue in two ways. "We are currently planning phase II studies on this issue and hope that the results we have found so far will be confirmed," says Frankenberger. This would really help COPD patients a great deal: specific vitamin A therapy would turn down mechanisms decomposing tissue in the lungs and inhaled particles could cause less damage.

On 8 March 2006 a European patent for an "Agent for Treating Illnesses of the Tracheobronchial Tract, Especially Chronic Obstructive Pulmonary Disease (COPD)" was granted for the invention.



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