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Monitoring of glucocorticoid therapy by assessment of $CD14^+CD16^+$ monocytes: A case report

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Abstract

Bronchiolitis obliterans with organizing pneumonia (BOOP) is a disease affecting small airways and alveoli. It is characterized by interstitial inflammation rich in foamy macrophages and by fibroblastic connective tissue expanding into the airway and alveolar lumen. We report herein on a 54-year-old male BOOP patient who was treated with glucocorticoids (GCs) and who over a 5-year period had three relapses. At diagnosis the patient showed elevated $CD14^+CD16^+$ monocyte numbers (85 cells/μl) and increased serum C-reactive protein (CRP) levels (29.4 mg/l). With GC therapy both parameters decreased within a few days. Diagnosis of relapse was preceded by a rise in $CD14^+CD16^+$ monocyte numbers and in CRP levels which again responded to GC treatment. We conclude that determination of $CD14^+CD16^+$ monocytes is a useful marker for monitoring of BOOP diagnosis and GC therapy.

Keywords: BOOP; Glucocorticoids; Monocyte subpopulations; CRP

Introduction

Bronchiolitis obliterans organizing pneumonia (BOOP) is a form of interstitial lung disease with distinctive clinical, radiological and histological findings. BOOP is characterized by intraluminal tissue plugs composed of granulation tissue in the lumen of the small airways extending into alveolar ducts and alveoli. This intraluminal tissue can obliterate the airways and lead to organization, i.e. fibrotic remodelling of the lung tissue. The patients typically present with a subacute onset of mild dyspnoea, a non-productive cough and patchy infiltrates on chest X-ray (Epler, 1992). In general idiopathic BOOP, the most common form of the disease, responds well to the treatment with glucocorticoids (GC); nevertheless, it might recur in one-third of the cases (White and Ruth-Sahd, 2007). BOOP can occur after a variety of different types of infectious pneumonias, including those caused by bacterial pathogens such as Chlamydia (Diehl et al., 1996), Legionella and *Mycoplasma pneumoniae* (Llibre et al., 1997). Drug-related BOOP is reported to occur after the use of several different types of medications, including antibiotics such as for example bleomycin as well as

Abbreviations: BOOP, bronchiolitis obliterans organizing pneumonia; CRP, C-reactive protein; GC, glucocorticoid.

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anti-seizure medications (Cordier, 2000; Rossi et al., 2000). Once the diagnosis has been established, therapy with prednisone is generally started at 1 mg/kg for 4–8 weeks and then tapered according to the clinical course (Epler, 1992).

GCs are widely used as oral and/or inhalative anti-inflammatory agents in a variety of diseases including asthma and COPD (Goldstein et al., 1999), but also in other chronic inflammatory situations such as rheumatoid arthritis (Buttgereit et al., 2005) and autoinflammatory diseases. GCs are steroid hormones, which can potently suppress immune response and inflammation. Their main mode of action is via the GC receptor-alpha (GCR-alpha), which is present in the cytosol as a homodimer, where it is bound to heat-shock proteins. When the lipophilic GCs enter the cell they bind to the receptor, leading to dissociation of the heat-shock proteins and to the translocation of the GCR into the nucleus. Here, the GC–GCR complex will induce gene expression by binding to cognate DNA sequences (Almawi and Melemedjian, 2002; Umland et al., 2002).

Inflammatory processes are often accompanied by elevated numbers of $CD14^+CD16^+$ blood monocytes (Fingerle et al., 1993; Horelt et al., 2002; Nockher and Scherberich, 1998), a subpopulation in human peripheral blood accounting for about 10% of all monocytes in healthy donors (Passlick et al., 1989). These cells have pro-inflammatory properties as they efficiently produce proinflammatory cytokines like TNF (Belge et al., 2002) and little of the anti-inflammatory cytokine IL-10 (Frankenberger et al., 1996). Randolph et al. (2002) describe the potential of the $CD14^+CD16^+$ monocytes to preferentially develop into dendritic cells. The $CD14^+CD16^+$ monocytes have been shown to respond to treatment with GCs (Fingerle-Rowson et al., 1998a; Dayyani et al., 2003) with a selective depletion in the first few days of GC application, sometimes accompanied by slight increase of the regular $CD14^{++}$ monocytes. *In-vitro* studies suggest that the depletion of the $CD14^+CD16^+$ monocytes is due to a process of apoptosis (Dayyani et al., 2003). The depletion of these cells may contribute to the anti-inflammatory action of GCs. Also the depletion may be a good indicator of the therapeutic efficacy of this type of drug.

In this case report, we followed a patient with BOOP over a period of 5 years, in order to assess whether determination of $CD14^+CD16^+$ monocytes in blood serves as an appropriate marker for monitoring the anti-inflammatory effects of oral GCs. Our study shows that the $CD14^+CD16^+$ monocyte numbers increase with disease activity and are depleted with institution of GC therapy. This suggests that determination of numbers of $CD14^+CD16^+$ monocytes may be useful in monitoring anti-inflammatory therapy with GCs.

Material and methods

Flow cytometry

For determination of $CD14^+CD16^+$ monocytes whole blood staining was performed as previously described (Dayyani et al., 2003). In brief, 100 µl of fresh EDTA-blood was admixed with directly conjugated monoclonal antibodies against human CD14 (clone My4-FITC, Beckman Coulter ♯6603511, Krefeld, Germany), CD16 (clone Leu11c-PE B73-1, BD sciences ♯332779, Heidelberg, Germany) and HLA-DR (PC5 conjugated clone ♯IM2659U, Beckman Coulter, Krefeld, Germany) and incubated for 20 min on ice. Red blood cells were then lysed according to the manufacturer's instructions using the Coulter Q-Prep device following the addition of 1 ml aqua dest. and 2 ml PBS/2% FCS. For absolute counting of monocyte subsets in whole blood 100 µl flow-count fluorospheres – containing 960 fluorescent beads/µl – (Beckman Coulter, ♯7547053) were added to the lysed blood suspension. Analysis was performed on a Coulter EPICS XL flow cytometer while gating on lymphocytes and monocytes in forward versus side scatter plots. After gating on CD14-positive and HLA-DR positive cells the $CD14^{++}$ and $CD14^+CD16^+$ monocytes were then determined in the CD14 versus CD16 histogram.

Calculation of the absolute monocytes count in whole blood

$$\begin{aligned}&CD14^{++}\text{or } CD14^+CD16^+ \text{ cells}/\mu l = \\ &\text{absolute number of } CD14^{++} \text{ or } CD14^+CD16^+ \text{ cells} \\ &\qquad \times 960 \text{ (beads}/\mu l) \\ &\qquad\qquad \text{absolute count of beads}\end{aligned}$$

C-reactive protein (CRP)

CRP in serum samples was determined in clinical diagnostic routine assay while using Dimension Flex reagent cartridge (Dade Behring ♯DF34, Ingelheim, Germany) according to the manufacturer's instructions at the day of blood collection. The RCRP (CRP Extended Range) method is based on a particle enhanced turbidimetric immunoassay (PETIA) technique. Latex particles coated with an antibody to C-reactive protein (AbPR) aggregate in the presence of CRP in the sample. The aggregates will absorb at 340 nm.

$$CRP + AbPR \rightarrow \text{Aggregate (absorbs at 340 nm)}$$

The increase in turbidity which accompanies aggregation is proportional to the CRP concentration. The concentration is determined by means of a mathematical function. The analytical sensitivity of RCRP method is