CORRESPONDENCE

Cytoimmunological monitoring after heart transplantation: Lymphocyte activation in *Pneumocystis carinii* infection

Cytoimmunological monitoring includes morphological differentiation (percentage of activated lymphocytes and lymphoblasts, plasmacytoid cells, and large granular lymphocytes) and immunological phenotype (CD4:CD8 ratio) of blood mononuclear cells. In this Journal we recently reported on the relevance of this assessment in patients after heart transplantation to diagnose rejection or infection.\(^1\) Lymphocyte activation (more than 5% activated lymphocytes or lymphoblasts) is indicative of infection or rejection. In this condition the presence of more than 15% large granular lymphocytes and a CD4:CD8 ratio of less than 1:1 indicates viral infection; the differentiation between rejection and bacterial infection is made by the presence of immature myeloid cells in the blood smear in case of bacterial infection.

Recently we were able to extend our previously published observations with the analysis of infection by the protozoan *Pneumocystis carinii*. Following heart transplantation two patients presented with this infection, which was documented by histological examination of an open lung biopsy specimen (Grocott staining) taken during the diagnostic work-up of pneumonia. Relevant data of cytoimmunological monitoring are presented in the figure. Both patients manifested a rejection episode of grade 2 (endomyocardial biopsy) before *P carinii* infection. In case 1 this was associated with lymphocyte activation in cytoimmunological monitoring, but in case 2 lymphocyte activation was not present at the time of this rejection. Case 2 also had severe cytomegalovirus (CMV) infection which had started eight weeks after transplantation documented by immediate antigen expression by peripheral blood granulocytes and viral culture. This infection was associated with lymphocyte activation, increased percentages of large granular lymphocytes in cytoimmunological monitoring (varying between 16 and 20%), and reversal of the CD4:CD8 ratio.

*P carinii* infection was reported at 30 weeks (case 1) and at 15 weeks (case 2) after transplantation. In both patients pneumocystis infection was associated with lymphocyte activation, with peak values of 5-3% (case 1) and 30-2% (case 2), respectively. During this infection there were no clinical or laboratory signs of rejection or viral or bacterial infection. Case 2 also presented with severe disease than case 1: higher lymphocyte activation in case 2 may have been related to the more severe aspects of the pneumonia. Treatment with prednisone in addition to conventional antibiotics was required. The pneumocystis infection and treatment in case 2 was followed one week later by reactivation of CMV documented by immediate antigen expression in blood granulocytes, without signs of associated disease. The peak level of lymphocyte activation may reflect both pneumocystis and CMV infection; therefore, the initiation of lymphocyte activation alone is the effect of pneumocystis infection. During pneumocystis infection, both patients showed normal values for percentages of large granular lymphocytes (< 15%), but presence of immature myeloid cells in the blood smear. In case 1 the CD4:CD8 ratio was < 1:0 before infection and remained low; in case 2 the CD4:CD8 ratio changed from values < 1:0 before infection to values of > 1:0 two weeks after infection.

*P carinii* infection is a rare complication of heart transplantation. Our data show that cytoimmunological monitoring might be a useful adjunct in the diagnosis of this infection; it is associated with lymphocyte activation (> 5% activated lymphocytes or lymphoblasts) in the isolated blood mononuclear cell population. The CD4:CD8 ratio, however, did not change substantially during pneumocystis infection. Large granular lymphocytes were not increased and immature myeloid cells did not appear in the blood. Thus the cytoimmunological monitoring criteria in pneumocystis infection resemble those used in rejection episodes. The criterion should be applied to exclude the possibility of cardiac rejection. In conclusion, in the diagnostic work-up of suspected pneumocystis infection following heart transplantation *P carinii* infection should be considered in case of lymphocyte activation in cytoimmunological monitoring.

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Follow up of cases 1 and 2 after heart transplantation. The time of *Pneumocystis carinii* infection lung biopsy specimen, the presence of rejection (grading in the endomyocardial biopsy specimen), CD4:CD8 ratio, and percentage of activated lymphocytes, including lymphoblasts, are shown.

Glycosylated proteins in lipofuscin

During the histochemical demonstration of histone proteins it was also observed that if EDTA slides were glycosylated with 300 M/l glucose in phosphate buffer (pH 8.9) at 37°C for one week they turned yellow, and with glucose-6-phosphate (G-6-P) at 56°C they turned brown. The strong colour was ascribed to what is called the "advanced Maillard browning product" following the Amadori rearrangement of the primary condensate of proteins and reducing sugars. The diffuse yellow colour appearing on the protein structures of formalin fixed, paraffin wax slides is strongly reminiscent of the colour of lipofuscin, which was suggestive of its formation.

The following experiments were carried out to support our assumption that lipofuscin contains glycosylated proteins. The fluorescence spectrum of the incubating glucose solution of the discoloured slides was recorded, on the one hand, and on the other 10 mg of human albumin (Serva, Germany) was incubated with 1.5 M/l glucose at 56°C. In