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 1.0 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 manifes-

ted 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 more 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%) and absence 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 appreciably 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 patients after 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 proteins¹ 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.² 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

the latter case, by day 4, a yellow, non-water soluble substance was precipitated. The fluorescence spectrum of the incubating solution of the slides and also that of the supernatant of the precipitated albumin-glucose are identical: the excitation maximum was 370 nm, that of emission, 435 nm.³ The fluorescence parameters of the brown incubating solution diluted with G-6-P were: excitation 410, 470 nm; emission, 525, 540 nm. These values agree with the maxima of fluorescence measured for lipofuscin *in situ*.⁴

Lipofuscin (and wear pigment) are known to be PAS, protein, performic acid-Schiff and lipid positive, fluorescent pigments chiefly found in tissue rich in glycogen or glucose (muscle, liver, nerve cells).⁵ The causes of PAS positivity and the fluorescence of lipofuscin have so far not been conclusively clarified.

The paraffin wax slides of the resin-like fluorescent substance forming from albumin-glucose *in vitro* are PAS positive, and give the Schmorl and NBT protein reaction. On the basis of our data we believe that besides lipoproteins and glycoproteins the glycosylated proteins are also considerable constituents of the lipofuscin-like pigments.

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Sensitivity of anti-HBc IgM kits and the diagnosis of acute hepatitis B

It was suggested by Morris that the presence of HBeAg and subsequent seroconversion to anti-HBe is a better indication of acute hepatitis B infection than the presence of anti-HBc IgM in cases where the patient is hepatitis B surface antigen (HBsAg) positive.¹ This conclusion is based on data showing that anti-HBc IgM concentrations remain high, even on dilution, for some months after HBeAg/anti-HBe conversion.

That anti-HBc IgM can be a useful diagnostic aid in active disease has been shown by other authors,^{2,3} and the detection of increased concentrations of anti-HBc IgM is normally associated with the acute phase of hepatitis B virus infection. One reason for the various opinions on the value of core IgM tests is the different cut-offs recommended by kit manufacturers of the assay methods used. We compared the performance of five commercial kits using a panel of sera with medium and low anti-HBc IgM concentrations and expressed the results in units of the Paul Ehrlich Institute reference preparation (U/ml). The effects of the different cut-offs of

Comparison of sensitivity, range, and detection of chronic cases in five commercial anti-HBc IgM kits

Kit name (manufacturer)	Cut-off antibody titre U/ml	Approximate antibody titre at assay response plateau U/ml*	No of positive or (retest) results on 20 sera from chronic HBV cases
Amerlite anti-HBc IgM Assay (Amerlite Diagnostics, Amersham)	250	800	0 (2)
Corzyme-M (Abbott Diagnostics)	290	800	0 (0)
Wellcozyme anti-HBc IgM (Wellcome Diagnostics)	60	350	4 (3)
Core IgM K EIA (Sorin Biomedica)	30	500	7*
Hepanostika anti-HBc IgM Micro ELISA (Organon Teknica)	15	510	12†

*Estimated using a panel of 22 samples. The anti-HBc IgM titres of these samples was determined from a calibration of Amerlite results and the Paul Ehrlich Institute reference preparation.

these tests can be seen in the results for a panel of 20 samples from chronic carriers (table).

It is evident that the antibody titre at the recommended cut-off for these assays varies by a factor of up to 20, and the assays where the cut-off is positioned at a low level give a higher detection rate with sera from chronic carriers. Using a panel of serial samples, we also showed that the mean period of positivity after the acute phase of infection varies from about two months for the less sensitive (high cut-off) assays to more than seven months with the most sensitive assay. The assay used by Morris was of high sensitivity and it would account for the finding of positive anti-HBc IgM results after the HBe/anti HBe seroconversion.

Detailed clinical studies have shown that an anti-HBc IgM concentration of > 600 U/ml is the best indication of the acute phase of HBV infection.² It is therefore important to consider not only the cut-off sensitivity but also the response range of the assay to be used. The assays have a variable upper level range (table) and some have limited or no ability to discriminate medium antibody titres from the more clinically important high (> 600 U/ml)

titres of antibody. It is these variations in sensitivity and response range that contribute to the divergence of opinions on the value of anti-HBc IgM in the diagnosis of acute hepatitis B. With an appropriate choice of cut-off, anti-HBc IgM concentrations remain positive only in the period of active disease (typically two months), and such a test is a useful vehicle for this application.

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ICAM-1 expression in normal liver

The paper by Smith and Thomas describes the distribution of the intercellular adhesion molecule-1 (ICAM-1) in a range of normal tissues,¹ including the liver on which we have also reported.² The results of their study are of great interest and provide a useful baseline for future studies on inflammatory conditions affecting the sites examined. The authors have interpreted our findings in a slightly misleading manner, however, and we would like to comment on two of the points they have raised.

Firstly, Smith and Thomas claim that their finding of positive staining for ICAM-1 in centrilobular hepatocytes is "in disagreement with" our study in which "predominantly sinusoidal staining for ICAM-1 was observed". While we would agree with the latter, we did in fact show that hepatocyte staining was commonly present in normal liver (donor specimens obtained at transplantation). Staining had a membranous pattern,

was generally faint, and tended to be dispersed fairly evenly throughout hepatic lobules, in contrast to the centrilobular distribution reported by Smith and Thomas. The clinical importance of ICAM-1 expression in normal liver cells is uncertain, and another recent study has shown no staining for ICAM-1 in normal hepatocytes.³

The second point concerns the expression of ICAM-1 in rejecting liver allografts. Although the distribution may be similar, the intense membranous staining pattern we observed in perivenular hepatocytes from rejecting livers was quite different from the faint staining reaction illustrated in fig 9 of Smith and Thomas's paper. The latter may, in part, be related to changes occurring after death, a point acknowledged by the authors in their discussion.

The expression of adhesion molecules is a dynamic process which can be modulated by many extracellular and intracellular events. Factors known to upregulate adhesion molecule expression include exposure to pro-