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Early infiltration of renal allografts with 27E10-positive macrophages and graft outcome

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Abstract Recently, we have demonstrated that acute cellular rejection is correlated with a massive infiltration of 27E10-positive macrophages. To examine the distribution of macrophage differentiation markers in the infiltrate in the very early post-transplantation period, two biopsies were taken intraoperatively, approximately 1 h following reperfusion, in each of 16 renal transplant recipients. One biopsy was taken for conventional histology and the other biopsy was snap-frozen. The sections were stained using an ABC indirect immunoperoxidase technique. A panel of monoclonal antibodies against three macrophage differentiation markers (27E10, 25F9 and RM3/1) was used to stain the sections. Using the early inflammation macrophage marker 27E10, there was an unexpected strong staining in 3 out of 16 biopsies. This severe infiltration of 27E10-positive macrophages with 10–20 macrophages per high power field (compared to 0–2 in others) was correlated in all cases with a poor outcome of the graft. All seven kidneys with no 27E10-positive infiltration showed a good function 6 weeks post-transplantation. The other macrophage markers, 25F9 and RM3/1, showed a less marked correlation with graft outcome. In conclusion, a massive infiltration of renal allografts with 27E10-positive macrophages 1 h post-transplantation may be a very early predictor of poor graft outcome.

Key words 27E10 · MRP8/MRP14 Monocytes/macrophages · Renal graft infiltration/rejection

Introduction

T cells are undoubtedly the cells responsible for alloantigen-specific graft rejection days or a few weeks following transplantation. However, neutrophils and monocytes play an alloantigen-unspecific key role in the early inflammatory response of the organ recipient only minutes and hours after transplantation. While over the last 2 decades, the interest of transplant immunologists has focused on T cell activation and T cell targeted therapy to prevent rejection, the role of macrophages, especially in the very first events following revascularization of the grafted kidney has sometimes been underestimated.

Macrophages fulfil multiple functions including T cell activation via presentation of the alloantigen in the context of the MHC class II antigens and additional interleukin-1 (IL-1) release, upregulation of adhesion

molecules on endothelial cells via secretion of IL-1, IL-6 and TNF alpha, thus enhancing the inflammatory response, and recruitment of further leukocytes by producing chemoattractants. Like activated T cells, activated tissue macrophages can be recognized immunohistologically by a change in their phenotype. Several monocyte activation and differentiation markers have been described so far. We were interested in the monocyte differentiation markers 27E10, 25F9 and RM3/1 described previously by Sorg et al [1, 2]. Only 15% of peripheral monocytes express 27E10. However, culture of monocytes or migration of monocytes into the tissue leads to an upregulated expression of 27E10. 27E10 has been shown to be present on macrophages in acute but absent in chronic inflammatory disorders [2]. RM3/1-positive macrophages seem to be associated with the healing phase and 25F9-positive macrophages with late or chronic inflammation.

Methods and patients

To examine the distribution of macrophage differentiation markers in the infiltrate in the very early post-transplantation period, two biopsies were taken intraoperatively approximately 1 h following reperfusion in each of 16 renal transplant recipients. One biopsy was taken for conventional histology and the other biopsy was snap-frozen. The sections were stained using an ABC indirect immunoperoxidase technique. A panel of monoclonal antibodies against three macrophage differentiation markers (27E10, RM3/1 and RM3/1) was used to stain the sections.

Results

Using the early inflammation macrophage marker 27E10, there was a strong staining in 3 out of 16 biopsies (Fig. 1). This severe infiltration of 27E10-positive macrophages with 10–20 macrophages per high power field (compared to 0–2 in others) was correlated in all cases with a poor outcome of the graft. All 13 kidneys with little 27E10-positive infiltration showed a good function 6 weeks post-transplantation. The other macrophage markers, 25F9 and RM3/1, showed a less marked correlation with graft outcome.

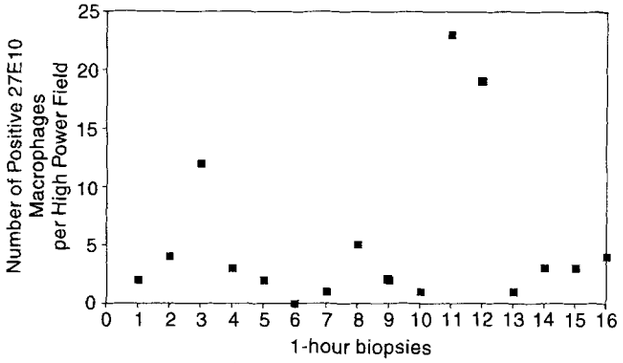

Fig. 1 27E10- (MRP8/14) positive macrophages in 1-h biopsies

Discussion

Our results showed that a massive infiltration of renal allografts with 27E10- (MRP8/MRP14 heterodimer) positive macrophages/neutrophils within 1 h post-transplantation may be a very early predictor of poor graft outcome, while the expression of RM3/1 and 25F9 did not correlate with graft outcome. In all inflammation models tested so far, 27E10-positive macrophages are the first to arrive at the lesion [1]. They have been described in acute heart [3], liver [4] and renal allograft rejection [5, 6]. 27E10 has, meanwhile, been cloned and biochemically characterized [2, 7].

Synonyms for this antigen are calprotectin, MAC 387 and 60B8 antigen and calgranulin. The 27E10 epitope is only revealed after non-covalent association of two proteins belonging to the S100 family of Ca^{++}-binding proteins, named migration inhibitory factor related protein 8 (MRP8) and MRP14, also known as p8/p14, leucocyte-derived protein 1 (L1) light/heavy chain or calgranulin A/B [7]. S100 proteins are abundant low molecular weight acidic proteins that were first described in 1965 by Moore in the nervous system. Their name derived from the unusual property of solubility in 100% ammonium sulfate. Each S-100 monomer protein contained two Ca^{++}-binding regions known as "EF-hand"s. Today, at least 10 Ca^{++}-binding proteins, including MRP8 and 14, with similar characteristics have been described in various cells and tissues. MRP8 is a 10.8-kDa protein of 93 amino acids and identical with the cystic fibrosis antigen. MRP14 is a 13.2-kDa protein and is the largest S100 protein with 114 amoni acids. Four isoforms have been described and it can be phosphorylated in position 113.

The exact function of this novel monocyte/neutrophil surface antigen is still unclear. *Extracellulary* it may be involved in activation and in migration, margination and diapedesis, leading to accumulaton of monocytes and neutrophils at inflammatory sites. *Intracellularly* it might be useful as a Ca^{++} buffer and probably inhibits casein kinase I and II activity, which might block phosphorylation of nuclear oncogenes, RNA polymerase II and

topoisomerase, thus, leading to terminal cell differentiation of neutrophils known to have a short life span.

MRP8 and MRP14 are abundant, mainly cytosolic proteins that are translocated to the surface during special phases of monocyte differentiation or activation. The MRP8/14 complex (27E10 epitope) is expressed exclusively on neutrophils and monocytes and on a few keratinocytes. The genes for MRP8/14 are located on chromosome 1. Our group [5, 6] and Steinhoff and Mues [3, 4] have already shown the massive infiltration of 27E10-positive cells in acute organ graft rejection. In the present study, documented that 27E10-positive macrophages can invade vascularized organs within 30 min. Moreover, this early massive infiltration seems to have predictive value for a poor outcome of the grafted kidney.

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