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CYTOMEGALOVIRUS ROLE IN INDUCING REJECTION REACTIONS IN HEART TRANSPLANTATION PATIENTS

A. HABOR [1] A. SIN [2] M.D. VARTOLOMEI[2]* I. CIUCA[3] H. SUCIU[4] M. TILINCA[2]

Abstract: *The study was performed on 45 patients with heart transplantation out of which 30 presented serologic cytomegalovirus infection. For histological study we used: hematoxylin eosin, van Gieson, Masson's trichrome, green methyl pironin stains. Immunohistochemistry was performed using ABC technique (avidin - biotin complexes or avidin - biotin) with the following monoclonal antibodies: CD8, CD4, CD20, CD68, CD31, CD45 RO, HLA-DR, and E13. In many of our cases cytomegalovirus infection was commonly associated with acute rejection. The presence of endothelial cells expressing HLA-DR antigens and subendothelial accumulation of activated T lymphocytes and macrophages, often in contact with endothelial cells, highlights the role of cellular immunity in the production of accelerated atherosclerosis lesions.*

Key words: *immunohistochemistry, acute rejection, cytomegalovirus, heart transplant, serology.*

1. Introduction

Cytomegalovirus infection is common in patients who benefit from transplant [13], [14]. We may refer to a primary infection, reactivation or reinfection through a virus transmitted by the graft or blood products used during surgical intervention. The presence of the virus in the endothelial cells of the heart vessels may be responsible for lesions of peripheral arterial disease, injuries playing an important role in producing chronic rejection [3], [9], [10]. Patients who have an organ transplant must undergo immunosuppressant treatment in order to prevent a rejection reaction [8]. The treatment has an inhibitory effect of immunological mechanisms and activates the action of cytomegalovirus. Instead, during viral infection, reducing immnunosupressive therapy may induce

[1] Department of Physiology, University of Medicine and Pharmacy Târgu-Mureş.
[2] Department of Cell and Molecular Biology, University of Medicine and Pharmacy Târgu-Mureş.
[3] "Prof.Dr. Octavian Fodor" Regional hepato-gastroenterology Institute of Cluj Napoca.
[4] Fifth Surgical Clinic, University of Medicine and Pharmacy Târgu-Mureş.
*Corresponding author: Senior Lecturer Mihai Dorin Vartolomei, e-mail: mihai.vartolomei@umftgm.ro

rejection reaction. Apparently, cytomegalovirus is a contributory factor of an undesirable rejection reaction [6]. Cytomegalovirus infection diagnosis is based on serological tests, histopathological examination, virus culture on human fibroblasts, detection of viral nucleic acids, immunohistochemistry and examination of the early and late viral antigens [5]. Cytomegalovirus seems to play an analogous role to that involved in the genesis of atherosclerotic lesions causing disturbances in endothelial cells: through functional alterations that are at the origin of a change in membrane permeability, favoring the accumulation of lipids in the vascular wall [4]. Endothelial cell activation means an aberrant expression of membrane molecules of the major histocompatibility complex molecules class I and II, adhesion molecules and the production of growth factors (platelet derived growth factor-PDGF and interleukin 1-IL1) [1], [2], [4], [7], [18].

2. Material and Methods

The study was conducted on 45 patients with heart transplantation performed at the Institute for Cardiovascular Disease and Transplantation,Târgu-Mures, between 1999 to 2014, from which 30 had serologic infection with cytomegalovirus. Cytomegalovirus seroconversion was defined as occurrence of specific anti cytomegalovirus IgM or IgG titers in serum. Serological tests were performed in duplication. For histological study we used the usual stains: hematoxylin eosin, van Gieson , trichrome Masson, methyl green pironin. Diagnosis protocol for acute rejection also included immunohistochemistry: ABC (avidin-biotin complexes or avidin-biotin) with the following monoclonal antibodies: CD8, CD4, CD20, CD68, CD31, CD45 RO, HLA-DR, E13, as previously described [11], [12],[15-17] (Table 1).

Monoclonal antibodies used Table 1

no.	Monoclonal antibody	Expression
1	CD8	Cytotoxic lymphocytes
2	CD4	Helper T lymphocytes
3	CD20	B lymphocytes
4	CD45Ro	T lymphocytes/
5	CD68	Macrophages
6	CD31	Vascular endothelium
7	E13	Cytomelovirus
8	HLA-DR	Major histocompatibility complex, class II

3. Results and Discussions

From 30 cases infected with CMV, 22 were men (73%) and 8 women (27%). The age of the patients ranged from 12 to 60 years. Histological examination performed on 147 slides revealed a case of arterial foam characterized by subendothelial accumulation of foamy histiocytes and intimal concentric thickening and three cases of inflammatory reaction with clustered polymorphonuclear neutrophils and focal myocytes necrosis, associated with cells with characteristic ,,owl eye" inclusions (Figures 1 and 2).

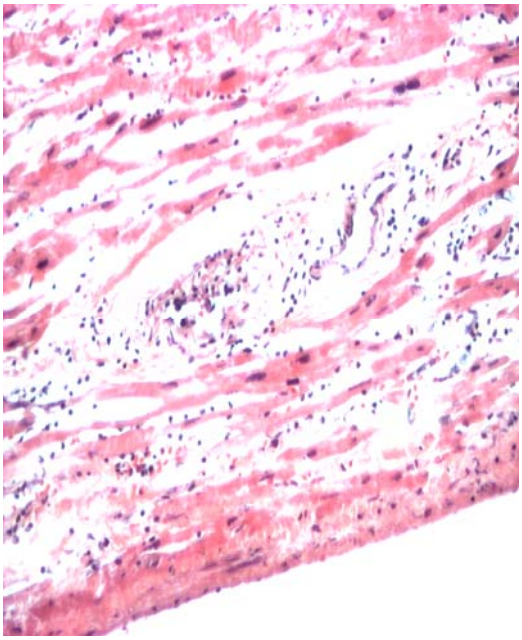

Fig. 1. *Small intramiocardic vessel. Endothelial lesion. Subendothelial foamy histiocytes; exocytose; chronic interstitial inflammatory infiltrate. Hematoxylin Eosin stain, x20*

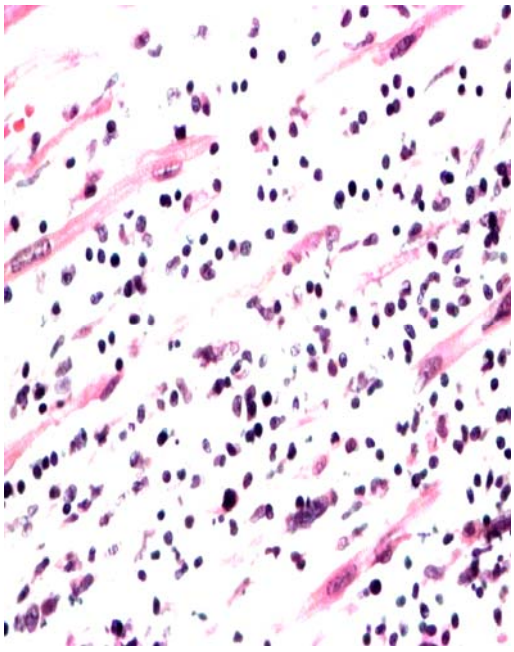

Fig. 2. *Acute rejaction reaction. Cytomegalovirus characteristics cells with "owl eye" inclusions. Difuse lymphoplasmocitic inflammatory infiltrate. Hematoxylin Eosin stain, x40*

Immunohistochemical study enabled the identification of different cell types that induce or determine accelerated arterial lesions: histiocytes with phenotype CD68, HLA-DR +, present in the intima and media of examined arteries (Figure 3).

Cytotoxic T lymphocytes (CD8 phenotype) accounted almost 50% of the lymphocytic population. Immunoassay was performed for early and late cytomegalovirus antigen detection, in one case with foamy arterial histiocytes and in the other 20 cases with intimal concentric thickening E13 marking was observed only in lymphocytic and histiocytic inflammatory cells, none of the cells were positive for late antigen marker.

Involvement of the CD4 lymphocytes is achieved through the release of cytokines and growth factors, whereas that of the CD8 lymphocytes by their specific action.

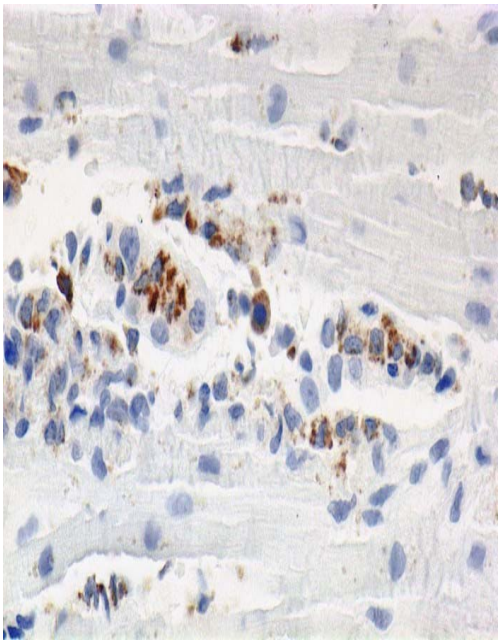

Fig. 3. *CD68 immunostaining macrophages „.2x, 4x, 10x*

The activation of endothelial cells is made through the aberrant membrane expression of the major histocompatibility complex (CMH class II), adhesion molecules and the secretion of growth factors (PDGF, IL-1). Macrophages and activated endothelial cells, present HLA-DR antigens that activate CD4 lymphocytes, cytokines and growth factors, triggering and maintaining the immunological process. The result is a proliferation of the smooth muscle cells [17].

In our study, CD8 lymphocytes accounted for over 50% of intimal lymphocytes. Ultrastructural and immunological studies with anti-perforin highlighted in literature also, have confirmed the cytotoxicity of these lymphocytes. Cytomegalovirus can damage the endothelial cells, causing functional disturbances manifested through an increase in cell membrane permeability, particularly for the accumulation of lipids in the vessels wall.

Cytomegalovirus infection plays a role in

inducing and realization of accelerated atherosclerosis lesions associated with chronic rejection of heart transplant, without being a direct correlation between infection and the patient failure. Only in two cases from our series of patients we could assign CMV infection as an aggravating factor.

From the clinical point of view we did not notice signs or symptoms of this infection, but typical features or signs of ischemic heart failure.

Measures to prevent cytomegalovirus infection in seronegative patients listed for transplantation would be transfusions only from sero-negative donors and sero-negative heart donor, still these measures are hard to achieve due to shortage of organs or in case of emergency interventions. In case of a CMV positive donor, usually unavoidable, preventive anti-CMV immunoglobulin treatment or the administartion of antiviral drugs in high doses would greatly reduce disease severity. In literature the possibility of administrating an antiviral vaccine with an attenuated strain (strain Towne) that would prevent the installation of severe forms of the disease is mentioned.

In the era of organ transplantation, cytomegalovirus has become a major problem of public health. Progress in early detection of the virus, as well as in establishing an advanced therapeutic protocol significantly improves the prognosis of this infection [4].

In future, in addition to serological marker tests in vitro examination of isolated strains for viral sensitivity tests should be implemented. Furthermore, new antiviral molecules are needed to be introduced in the therapeutic protocol, specific for CMV.

In conclusion, cytomegalovirus induces release of interferon gamma in endothelial cells by activated T-lymphocytes, which alter the expression of major histocompatibility antigen complex on the surface of the smooth muscle cells and endothelial cells, favoring the growth of immune reaction and acute rejection in heart transplant patients. In many of our cases cytomegalovirus infection was commonly associated with acute rejection.

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Correspondence: Senior Lecturer Mihai Dorin Vartolomei, Department of Cell and Molecular Biology, UMPh Târgu-Mureş, Str. Gh. Marinescu 38, Tel: +040741728809, E-mail – mihai.vartolomei@umftgm.ro

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