

Immunopathological Heart Modifications in Experimental Trichinellosis as a Model of Helminthic Myocarditis

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Abstract

Following experimental infection with *Trichinella spiralis*, isolated and perfused rat hearts underwent spontaneous and severe arrhythmic episodes in coincidence with tissue and blood hypereosinophilia (21 days post-infection). At this time, functional parameters such as coronary flow and end-systolic-LVP consistently decreased. The histological data showed, at 21 days, a large amount of degranulated mast cells in the perivascular zone of the myocardium but no significant changes in myocardial structure. Also at 48 days, when the eosinophils both in blood and in the cardiac interstitium decrease, rhythm disturbances frequently occurred, accompanied in some cases by an evident trend to dilatation, without relevant histological change in myocardial structure. IgG complexes were found in the myocardial interstitium, especially at 48 days of infection, whereas parasitic antigens were absent, contrary to other helminthic myocarditis models. We suggest an immunopathological mechanism for the myocarditis model described. The interaction between eosinophils, their products and mast cells are presently under investigation.

Key words: myocarditis, eosinophils, mast cells, trichinellosis.

BAM 5 (3): 223-230, 1995

Parasitic diseases have among their characteristics a great variability in cardiologic alterations, e. g. arrhythmias, myocarditis [6, 11, 18] and different cardiomyopathy patterns (i.e. Chagas' disease). Rhythm and haemodynamic changes observed in Chagas' disease may be attributed to vascular disorders (defect in arterial flow and/or lymphatic drainage), which would lead to substitutive endomyocardial fibrosis and myocardial damage typical of the chronic state of the disease [11, 17].

Human trichinellosis is also characterised by heart involvement, which may vary from fatal arrhythmias to severe myocarditis [12]. The myocarditis observed during human infection may be distinguished from others of different origin (e. g. viral) by the presence, in addition to mononuclear cells, of high eosinophil numbers in perivascular and interstitial infiltrates as well as around foci of damaged tissue [20]. This suggests that inflammatory cells such as neutrophils and eosinophils could damage myocardial cells with a reduction of heart function which occurs mainly in the late phase of infection [15]. However, the mechanisms responsible for myocardial damage in trichinellosis are far from being clarified.

In trichinellosis, heart involvement and especially arrhythmias are generally considered as due to the passage of migrant larvae in this organ [7]. These, however, are not able to encyst in myocardial cells. Among the hypotheses advanced to explain this phenomenon, the inability of myocardium cells to form "nurse cells" appears the most probable [19].

Very recently, however, in experimental rat infections irreversible arrhythmic episodes were observed, not only at 15 and 21 days, but also at 48 days of infection [13], when parasite migration is completed [7]. At this time of infection blood eosinophil levels were still high, suggesting that these cells could trigger immunopathological processes which might play a role in myocardial damage. *In vitro* studies have shown that the main degranulation products of eosinophils, such as the Major Basic Protein, have a cytotoxic effect against a wide variety of mammalian cells [5].

The aim of this study is to describe the modifications which affect the myocardium in experimentally infected animals, with particular attention to histological and immunopathological alterations, to better clarify the possible

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mechanisms which underlie trichinellosis and other helminthic myocarditis.

Materials and Methods

Infection of experimental animals

Sprague-Dawley male rats 6 weeks aged were orally infected with 3,000 *Trichinella spiralis* (MSUS/WG/65/ISS51) infective larvae obtained as previously described [2]. Briefly, infected rats were sacrificed after at least 30 days from infection; their carcasses were minced and digested by agitation with a magnetic stirrer in an artificial gastric juice (1% HCl and pepsin). At the end of the digestion larvae were progressively concentrated by sedimentation at 1 g, counted and evaluated for their viability.

Experimental groups

They were represented by groups of at least 4 animals: 1) uninfected (controls); 2) infected at 21 days; 3) infected at 30 days 4) infected at 48 days and 5) infected at 140 days.

Histology

At the end of perfusion or after a simple wash-out with physiological solution the hearts were allowed to spontaneously stop beating, fixed in formalin and then paraffin-embedded in 58C Paramatt (GURR). Sections of 3µ, obtained by a rotative microtome (Reichert-Jung 2055 autocut), were stained with hematoxylin-eosin for myocardial structure evaluation; infiltrate composition was studied by means of Mann-Dominici staining (specific for "extravasal blood"), which resulted particularly useful for eosinophil detection (in brilliant red). Alcian-blue staining according to Lison [10] was performed to identify mast cells and their products, rich of acid mucopolysaccharides (in bluish-green) and, finally, toluidine blue (pH 0.5), which stains mast cells granules brilliant red to purple because of metachromatic acid mucopolysaccharides.

Evaluation of IgG immune complexes

Hearts which did not undergo perfusion were generally used. The presence of IgG immune-complexes in the myocardial tissue was evaluated with a direct immunofluorescence test using a FITC-conjugated polyclonal antibody specific for rat IgG (Dako Co., Santa Barbara, Ca), incubated at 1:20 dilution with frozen heart sections from controls or from animals at 30, 48 and 140 days of infection.

Evaluation of parasitic antigens

To detect *Trichinella spiralis* antigens, deparaffinized sections of rat heart at 21 and 48 days of infection were incubated first with hydrogen peroxide to inactivate endogenous peroxidase, then with the mouse monoclonal PG6B1 antibody (kindly provided by Dr. C. Marini, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche) which recognises proteins of 48-53 KDa present in the *T. spiralis* muscle larvae. The indirect immunoper-

oxidase technique was performed according to the following protocol: PG6B1 diluted 1:1000 at room temperature (r.t.) for 30 min; biotinylated anti-mouse IgG at r.t. for 30 min; preformed avidin-biotin peroxidase complex (Vector Labs., Burlingame, Ca) at r.t. for 30 min. Reactions were developed with 3,3' diaminobenzidine plus hydrogen peroxide. As positive controls, diaphragm sections of *T. spiralis* infected rats were used.

Heart perfusion

Under deep ether anaesthesia, the hearts from 21, 30, 48 and 140 days infected animals were quickly removed from the chest, the aorta was retrogradely cannulated and perfused, at least 90 min, using a Langendorff apparatus with non-recirculating Tyrode solution at 37°C gassed with 95% O₂+5% CO₂ at constant flow (13 cc/min) by means of a peristaltic pump. The coronary resistance (CR) was continuously monitored by a pressure transducer applied to the aortic cannula. The isovolumetric left ventricular pressures (EDLVP and ESLVP) were measured with a latex balloon inserted into left ventricle and connected via a water-filled Teflon tube to a pressure transducer; the ECG was recorded by means of bipolar wick electrodes.

The hearts were paced at a rate of about 4/sec.

Results

Histological findings

Interstitial infiltrates of inflammatory cells such as eosinophils and mononuclear cells are more prominent at 21 days from infection: their distribution is either in small foci or in perivascular groups (Fig. 1). We did not observe areas of marked myocardial damage; however, conspicuous oedematous areas were observed sometimes close to inflammatory infiltrates which led to fiber disarrangement (Fig. 1). No alterations, in terms of myocardial structure and organelles (i.e. mitochondria), was revealed by electron microscopy at any time of infection (data not shown).

Mast cell evaluation

Concerning the distribution and degranulation degree of the mast cells found in myocardial tissue, we considered these parameters at 21 days when blood and tissue eosinophils reach a peak (acute infection) and at 48 days when both are sloping down to control values (late phase), according to previous results [13]. The occurrence of highly degranulated cells is 67% of the total in 21 d.i., 23% in 48 d.i. and 20% in control animals. Furthermore, in 21 d.i. group highly degranulated mast cells were more frequent in perivascular zones than far from vessels (Fig. 2A). On the contrary, at 48 d.i. the occurrence of degranulated mast cells (including the perivascular ones) went down to control values (Fig. 2B).

Functional parameters

CF was significantly decreased, after 30 min of perfusion, in 21 d.i. in comparison with controls (63% of controls) and ESLVP, at this infection time was 50% of

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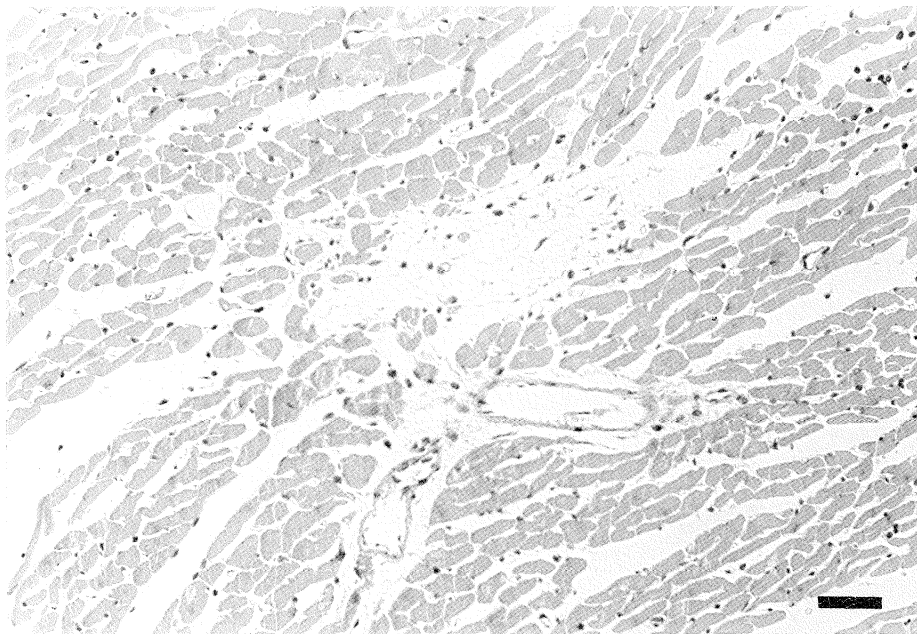


Figure 1. Myocardium section from a rat at 21 days of infection. Diffuse inflammatory cell infiltrate with areas of fiber disarrangement (Mann-Dominici' staining, calibration bar = 42 μ m).

controls. At other infection times hearts did not show any significant change relative to controls, with the exception of 48 d.i. in which a consistent decrease both in ESLVP and EDLVP was observed in some hearts, suggesting that myocardium at this time has the tendency to dilate.

A follow-up of infection (extended up to 140 d.i.) did not show any significant change, in terms of physiological parameters, with the exception of one case of ventricular fibrillation.

Evaluation of IgG-immune complexes

They are already present in the myocardium, starting from 30 d.i. However, the fluorescence, both cellular and interstitial, is particularly evident in 48 d.i. animals (+/++) (Fig. 3) when compared to controls () (Fig. 4). After 140 d.i. IgG are still present even if the fluorescence is more attenuated (+).

Evaluation of parasitic antigens

No myocardial tissue labelling was observed in rats at 21 and 48 d.i. with parasite-specific monoclonal antibody which in positive controls caused a strong reaction in the whole parasite structure (Fig. 5).

Discussion

The mechanisms responsible for heart involvement during trichinellosis are far from being clarified and generally a great importance is attributed to the passage of parasites through the capillary vessels of heart [7]. Very recently, however, in experimental infections in rats irreversible arrhythmic episodes were observed not only at 15 and 21

days but also at 48 days of infection [13] when parasite migration is completed [7]; at this time of infection blood eosinophil levels were still high, suggesting that these cells could trigger immunopathological processes which might play a role in the myocardial damage.

In the last few years, the importance of the rôle of eosinophils *in vivo* in protective immunity against *Trichinella spiralis*, as in other helminth infections, has been dramatically reduced; in fact, infected mice, in which eosinophilia was completely suppressed by blocking Interleukin 5, had the same worm burden as control animals [8]. This intriguing result, which decreases the importance of all *in vitro* studies performed in the last fifteen years (describing a cytotoxic effect of eosinophils against a great number of helminthic migrant stages) shifts the interest of researchers to the possible immunopathological effects, in different organs, of the high levels of blood eosinophils which are present during helminth infections.

In experimental infection in mice with the helminthic nematode *Toxocara canis* histopathological alterations of myocardium are characterised by the formation of focal histiocyte and eosinophil infiltrates which evolve into necrotic debris containing granulomata [3]; furthermore, Schaffer and coll. [18] in experimentally infected rats observed a decrease in cardiac performance coincident with increase in blood eosinophils, with a reduction of cardiac work of about 25%. Myocardial dysfunction was also observed after exposure of perfused uninfected hearts to activated eosinophils. The hearts perfused with activated eosinophils showed also important histological al-



Figure 5. Immunoperoxidase stain of a diaphragm sample of rat infected with *T. spiralis* at 40 d.i. incubated with the monoclonal antibody PG6B1. The antigen of 53kDa recognised by the monoclonal antibody is present in the whole parasite structure. It is possible to note the complete absence of staining in the muscle tissue (calibration bar = 17µm).

Acknowledgements

The Authors wish to acknowledge the help of Dr. P. Belati-Biagiotti for immunohistochemical studies and the technical assistance of M. Bettini.

This work was partially funded by the Italian Ministry of University (60%).

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