Immunology 1988 **64** 725–731

Immunohistochemical detection of deposits of eosinophil-derived neurotoxin and eosinophil peroxidase in the myocardium of patients with Chagas' disease

H. A. MOLINA & F. KIERSZENBAUM *Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan, U.S.A.*

Accepted for publication 22 March 1988

SUMMARY

An immunohistochemical study of eosinophil distribution in the inflammatory cell infiltrates of four different types of myocardial lesions associated with Chagas' disease—caused by *Trypanosoma cruzi*—showed larger numbers of these cells in areas presenting tissue necrosis and degeneration, most notably in patients with the most severe myocarditis from a histopathological stand-point. Using antisera specific for human eosinophil-derived neurotoxin or eosinophil peroxidase, we detected deposits of these secretion products on myofibres and in the interstitium of chagasic myocardium displaying necrosis and degeneration but rarely in other types of lesions. These deposits were not detectable in the myocardium of non-chagasic patients who had died from myocardial infarction (acute or in the scarring stage) or myocarditis secondary to bacterial endocarditis. When human eosinophil-derived neurotoxin was incubated with myoblast monolayers there was significant cell injury, detachment and lysis. These effects were abrogated by yeast RNA, added as a competitive ribonuclease substrate, and inhibited by the ribonuclease inhibitor RNasin, suggesting that the ribonuclease activity of the eosinophil-derived neurotoxin was involved in the effect. These results suggest a link between eosinophil infiltration and necrosis in chagasic myocardial lesions and point to EDN, and perhaps other toxic eosinophil secretion products, as possible mediators of tissue damage.

INTRODUCTION

Tissue lesions developing during Chagas' disease—caused by the protozoan *Trypanosoma cruzi*—are typically infiltrated by lymphocytes, monocytes, macrophages, neutrophils and eosinophils (reviewed by Romaña, 1963; Andrade & Andrade, 1979; Andrade, 1985) but the precise role(s) played by these cells at chagasic lesion sites is not known. *In vitro* experiments have shown that human neutrophils (Villalta & Kierszenbaum, 1983), eosinophils (Villalta & Kierszenbaum, 1984a; Kierszenbaum, Villalta & Tai, 1986), monocytes and macrophages (Villalta & Kierszenbaum, 1984b) can take up and destroy amastigotes (tissue forms) of *T. cruzi*, suggesting a potential for these cells for participation in host defence. However, a quantitative study of inflammatory cell distribution in different types of chagasic lesions has recently uncovered a link between eosinophil infiltration and the more severe, necrotic type of chagasic myocardial lesion (Molina & Kierszenbaum, 1987). Although Köberle (1968) described an occasional accumulation of eosinophils in heart lesions from some chagasic patients and Rowland & Sibley-Phillips (1984) reported increased eosinophil levels in the bone marrow of *T. cruzi*-infected mice, not much additional attention has been given to the role that this inflammatory cell type may play in the course of Chagas' disease. In the present study, we used an immunohistochemical method to both enumerate eosinophils and identify eosinophil-secretion products in different types of chagasic myocardial lesions. It will be shown in this paper that eosinophil infiltration is particularly associated with the necrotic, degenerative chagasic type of chagasic myocardial lesion in which deposits of toxic eosinophil secretion products were found. One of these products, the eosinophil-derived neurotoxin (EDN; also known as eosinophil protein-X), was found in this work to damage heart cells *in vitro*.

Abbreviations: CL, myocytolysis; DN, myocarditis with degeneration and necrosis of myofibres; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EPO, eosinophil peroxidase; IF, interstitial fibrosis; MBP, eosinophil granule major basic protein; MM, minimal myocarditis and essentially preserved myofibres.

Correspondence: Dr F. Kierszenbaum, Dept. of Microbiology and Public Health, Michigan State University, East Lansing, MI 48824, U.S.A.

MATERIALS AND METHODS

Study cases

The tissues used in this investigation were obtained from one patient with acute and nine patients with chronic Chagas'

disease. The hearts from these patients were obtained, within 4 hr of death, during autopsies performed at the Ramos Mejia Hospital, Buenos Aires, Argentina. Prior patient consent was received upon admission to the hospital and further authorization was given by next of kin after death. The clinical history of the acute case included parasite isolation from blood by xenodiagnosis and electrocardiographic alterations (prolonged PR and ST-T abnormalities), such as seen in patients with myocarditis. The clinical histories of all of the chronic cases included a positive complement-fixation test for anti-*T. cruzi* antibodies. A positive indirect immunofluorescence test was also available for four of these cases. All 10 patients presented clinical symptoms consistent with cardiomyopathy, and X-radiograms revealed cardiomegaly in eight of the chronic cases. Electrocardiographic abnormalities (right bundle branch block, left anterior hemiblock, atrioventricular block and/or ventricular premature beats) were recorded in eight of the chronic cases). The single chronic patient who did not show electrocardiographic abnormalities presented, instead, cardiomegaly with apical aneurysm and thrombosis in the right atrium and left ventricle. As controls we used normal heart tissues from two individuals who had died from cerebral-vascular accidents, two patients who had died from acute myocardial infarction, two patients with myocardial infarction in the scarring stage who had died with arrhythmias, and two patients with myocarditis secondary to bacterial endocarditis, also dying from their cardiac disease.

Heart tissue processing

Two to three tissue specimens were obtained from the left ventricle of each patient, fixed in 10% formaldehyde and embedded in paraffin. For immunohistochemical studies, 6- to 7-μm thick tissue sections were stained after incubation with rabbit antisera specific for human EDN or eosinophil peroxidase (EPO), kindly provided by Dr G. J. Gleich, Mayo Clinic, Rochester, MN. The antigens used to prepare these sera were purified, as described elsewhere (Ackerman *et al.*, 1983; Gleich *et al.*, 1986; Slifman *et al.*, 1986), to homogeneity in sodium dodecyl sulphate polyacrylamide gels under reducing conditions (Gleich *et al.*, 1986; Slifman *et al.*, 1986; S. L. Dunnette and K. Hamann, personal communication). Phosphate-buffered saline solution, pH 7·2 (PBS), was used for all washings, solutions and dilutions and, unless otherwise stated, incubations took place at room temperature for 30 min. Each tissue section was incubated with 15% H_2O_2 to inactivate endogenous peroxidase. After washing three times, the sections were incubated with 0·1% trypsin (Sigma, St Louis, MO), washed again and incubated with normal mouse serum diluted 1/20. The sections were then washed and incubated at 37° for 2 hr with either anti-EDN or anti-EPO serum diluted 1/40. After washing, the sections were incubated with horseradish peroxidase-conjugated swine anti-rabbit Ig (Accurate Chemical, Westbury, NY) diluted 1/50 and washed again. For colour development, we used 0·3 mg/ml diaminobenzidine in 0·05 M Tris-buffer, pH 7·5, containing 0·003% H_2O_2. For control purposes, tissue sections received the same treatment, except that normal rabbit serum was used instead of an anti-eosinophil-protein serum. No positive staining was observed in any of the controls. In all cases, the sections were counterstained with haematoxylin.

Types of histological patterns in chagasic heart tissue

Histological patterns were defined according to the following criteria: (i) areas with myocarditis associated with degeneration and necrosis of myofibres (referred to in the text as DN); (ii) areas with minimal myocarditis and essentially preserved myofibres (MM); (iii) areas with interstitial fibrosis (IF); and (iv) areas with myocytolysis (CL). In applying these criteria, myofibre degeneration was defined by the presence of vacuolization and/or the occurrence of partial coagulative changes in the cytoplasm. Necrosis was defined by the presence of complete coagulative changes in the cytoplasm of muscle cells, myofibre fragmentation or disintegration, and phagocytosis of cellular debris by mononuclear cells. Myocytolysis was, as described by Schlesinger & Reiner (1955), characterized by progressive lysis of myofibrils, seldom accompanied by inflammation. To establish the presence or absence of each of these histologic patterns, six sections were screened for each tissue specimen.

Histopathological evaluation of chagasic myocarditis

The overall severity of the myocarditis of each patient was semi-quantified histologically. We assigned 4+ to the acute chagasic patient, who had the most extensive necrosis of all 10 study cases; 3+ to the patients with well-defined myocardial lesions and relatively large areas of damage; 2+ to patients with multifocal degenerative and necrotic lesions in which some foci converged to form a large lesion; and 1+ to patients with degenerative and necrotic lesions affecting isolated muscle fibres. Patients showing myocarditis without necrosis and myocardial degeneration were classified as (−).

In all cases, not less than 200 microscopic fields (× 1000) were screened in each area examined and the number of eosinophils with the appropriate staining was recorded. The results were expressed as the average number of eosinophils per 100 fields. Because parasites are often not detectable in diseased tissues from chronic chagasic patients (reviewed by Andrade & Andrade, 1979), and nine of our 10 study cases were chronic, parasite counts were not taken.

Toxic effect of EDN on myoblasts

The EDN used in this work was a generous gift from Dr G. J. Gleich. Its purification to homogeneity (Gleich *et al.*, 1986; Slifman *et al.*, 1986) and the ribonuclease activity of this preparation (Slifman *et al.*, 1986) have been described elsewhere. Two methods were used to determine the toxic effects of EDN on rat heart myoblasts (RHM). In one of these methods, RHM cultures, maintained as described elsewhere (Lima & Kierszenbaum, 1982), were plated at 4×10^4 cells/well in medium containing 60 μCi/ml $Na_2{}^{51}CrO_4$ (specific activity 400 Ci/g Cr; ICN Radiochemicals, Irvine, CA) in 96-well (flat-bottomed) microculture plates. Replicate plates were incubated at 37° for 20 hr in a 10% CO_2 atmosphere saturated with water vapour, washed three times with Eagle's minimal essential medium (Gibco, Grand Island, NY) and incubated further with 100 μl of 1×10^{-4} M solution of EDN in the same medium under the same conditions for 6 hr. Control cultures were incubated with medium alone. To explore the mechanism of action of EDN, we incorporated 0·5 U/μl RNasin™ (Promega, Madison, WI) or 4 mg/ml yeast RNA (Sigma) into the culture medium. The fluid phase was then removed and centrifuged (350 ***g***, 10 min). Radioactivity was determined in the supernatants and

Table 1. Eosinophil infiltration and presence of deposits of EDN and EPO in chagasic myocardial lesions with histologic pattern DN

Severity of myocarditis	Eosinophils detected with*		EDN and EPO deposits	
	Anti-EDN	Anti-EPO	Myofibres	Interstitium
4+†	290	312	Present	Present
3+	228	235	Present	Present
3+	189	178	Present	Present
3+	161	165	Present	Present
2+	35	40	Present	Absent
2+	37	21	Present	Absent
1+	14	15	Present	Absent
1+	3	4	Absent	Absent

* Results are expressed in terms of the average number of positively stained eosinophils per 100 fields.

† Patient with acute Chagas' disease.

also in lysates of the myoblasts that remained attached to the wells. The latter were prepared by incubating the attached cells with 100 μl of 1% Triton X-100 at 37° for 1 hr. Spontaneous release of radioactivity was determined in the supernatants (350 ***g***, 10 min) from cell cultures incubated with medium alone. Total releasable radioactivity was measured in cultures receiving 100 μl of 1% Triton X-100. For a measure indicative of cell lysis we used the equation:

$$\% \text{ lysis} = \frac{\text{test c.p.m.} - \text{spontaneous c.p.m.}}{\text{total c.p.m.} - \text{spontaneous c.p.m.}} \times 100.$$

Radioactivity contained in cells that became detached during the experiment was calculated by the equation:

$$\% \text{ detachment} = \frac{\text{total c.p.m.} - \text{supernatant c.p.m.} - \text{monolayer c.p.m.}}{\text{total c.p.m.}} \times 100$$

where supernatant c.p.m. was the radioactivity of the supernatant obtained after centrifugation (350 ***g***, 10 min) of the fluid phase.

In the other method, RHM monolayers attached to the 3-mm diameter wells cut on Teflon-coated glass microscope slides (Cel-Line, Newfield, NJ) were incubated with 15 μl of medium alone or containing EDN at 37° for 6 hr. The cultures were then washed with medium, fixed with absolute methanol and stained with Giemsa. At least 150 fields (× 1000) were screened in each well. All conditions were tested in duplicate or triplicate. The results were expressed as the average number of cells per 100 fields ± SD.

RESULTS

Presence of eosinophils and eosinophil secretion products in heart tissue areas with histological pattern DN, MM, IF or CL

Antisera specific for EDN or EPO were used to immunohistochemically identify eosinophils in areas of the examined heart tissue sections. As can be seen in Table 1, the number of eosinophils in areas with histological pattern DN were larger in patients whose myocarditis had been evaluated as being the most severe from a histopathological stand-point. The average numbers of EDN- and EPO-positive cells determined in adjacent sections were comparable in all cases. This similarity indicated that the variation in the eosinophil contents of

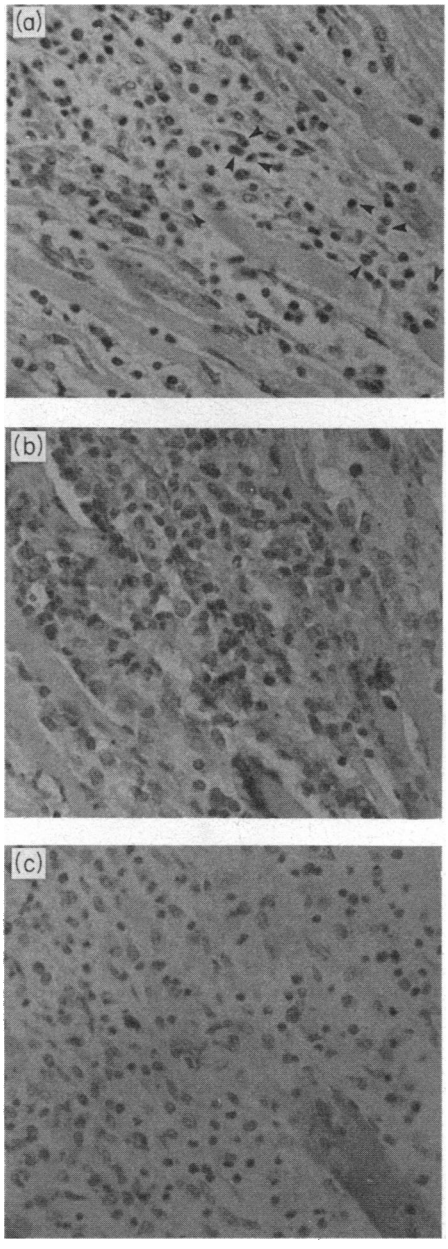

Figure 1. Eosinophil infiltration in chagasic tissue areas showing myocarditis with degeneration and necrosis of myofibres. To be noted, these photographs represent an original microscopic magnification of × 400 whereas × 1000 microscopic fields were screened to obtain the data shown in the tables. (a) DN area in a tissue section from the acute patient (myocarditis 4+) stained with haematoxylin and eosin. The arrowheads point to eosinophils. (b) Section stained immunohistochemically using rabbit anti-EDN serum (counterstained with haematoxylin). Note the numerous eosinophils stained with the typical brown colour produced by the peroxidase-catalized reaction. (c) DN area of a similar tissue section incubated with normal rabbit serum instead of rabbit anti-EDN.

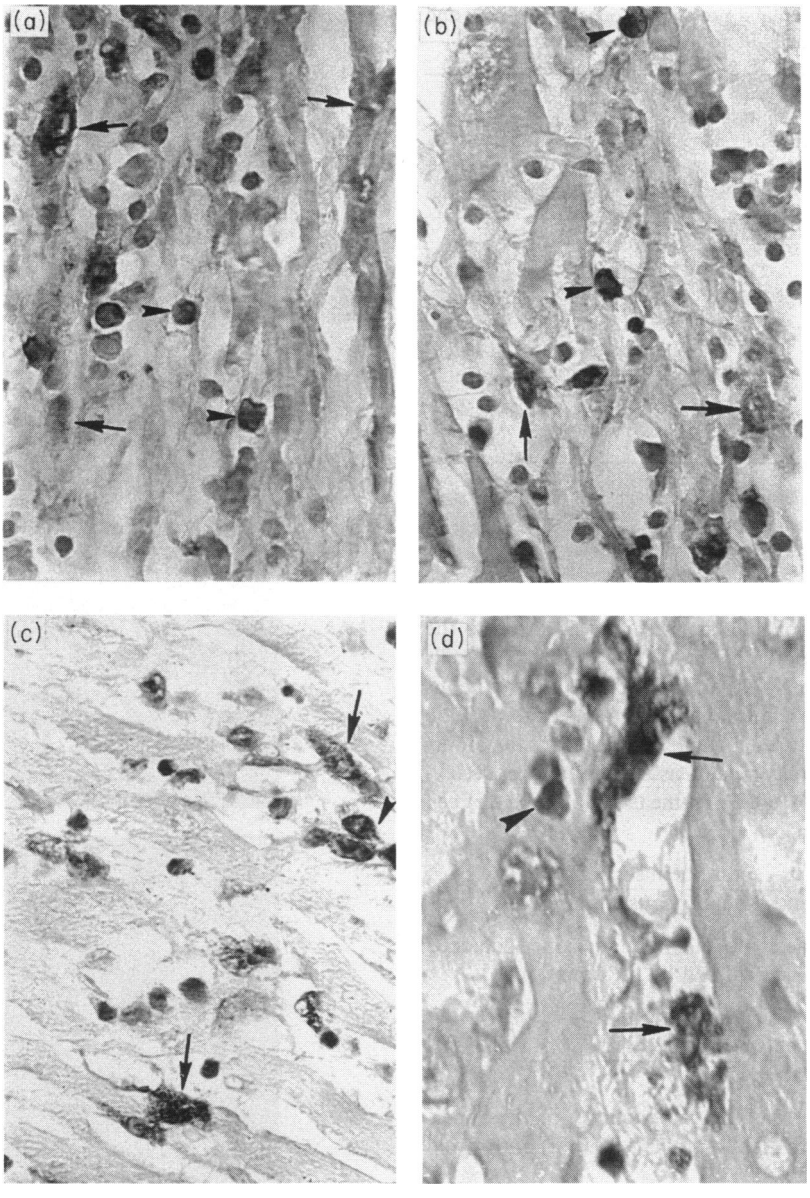

Figure 2. Identification of eosinophil-secretion products in chagasic tissue areas showing myocarditis with degeneration and necrosis. (a) Deposits of EDN in tissue from the acute chagasic patient. (b) Deposits of EPO in tissue from the acute chagasic patient. (c) Deposits of EDN in tissue from a chronic chagasic patient (myocarditis 3+). (d) Deposits of EPO in tissue from a chronic chagasic patient (myocarditis 3+). Arrows point to deposits of eosinophil granule material. Some eosinophils are indicated with arrowheads. In all cases, the original magnification was ×400.

adjacent tissue sections was insignificant. Illustrated in Fig. 1 is the comparatively large number of eosinophils, with their characteristic haematoxylin and eosin (Fig. 1, a) and immunohistochemical (Fig. 1, b) staining that was found typically in DN areas of chagasic myocardial tissue. Closely adjacent sections, subjected to a control immunohistochemical staining procedure in which normal rabbit serum substituted for anti-EDN or anti-EPO, produced negative results (Fig. 1, c).

Deposits of EDN and EPO were found both on myofibres and in the interstitium of DN areas (Table 1, Fig. 2). These deposits were most pronounced in the DN lesions of the acute case (Fig. 2a and b). Among the chronic cases, those with the most severe myocarditis (Fig. 2c and d) presented heavier deposits of EDN and EPO than those with milder myocarditis.

Eosinophil infiltration in lesions with histologic pattern MM was not as marked as in DN areas (Table 2). In contrast, eosinophil infiltration was minimal or undetectable in areas with patterns IF or CL. Deposits of EDN or EPO were not detectable in areas with MM, IF or CL, with the sole exception of one chronic case in which extracellular EDN and EPO were present in a single IF area.

Only two of the 10 study cases, both with chronic Chagas' disease, were classified as (−), i.e. presented myocardial inflammatory infiltrates without necrosis and degeneration of

Table 2. Eosinophil infiltration and presence of deposits of EDN and EPO in chagasic myocardial lesions with histologic pattern MM, IF or CL

Severity of myocarditis	Eosinophils detected with Anti-EDN* MM	IF	CL	Anti-EPO* MM	IF	CL	EDN and EPO deposits Myofibres	Interstitium
4+†	80	NP‡	NP	82	NP	NP	Absent	Absent
3+	33	12	NP	41	7	NP	Absent	Absent
3+	3	9	NP	5	3	NP	Present§	Absent
3+	9	10	NP	11	7	NP	Absent	Absent
2+	12	NP	7	8	NP	9	Absent	Absent
2+	9	NP	NP	6	NP	NP	Absent	Absent
1+	6	3	NP	3	1	NP	Absent	Absent
1+	1	NP	2	1	NP	0	Absent	Absent
(−)	1	0	NP	0	0	NP	Absent	Absent
(−)	1	NP	0	1	NP	0	Absent	Absent

*, † See footnotes to Table 1.

‡ NP, this histologic pattern was not present. Six sections from each tissue specimen were examined before a histologic pattern was considered to be absent.

§ These deposits of EDN and EPO were present in some IF areas.

Table 3. Effects of RNasin and yeast RNA on EDN toxicity for RHM*

Tested materials	% lysis (%R)†	% detachment (%R)†
Medium alone	0	0
Yeast RNA, 4 mg/ml	0	0
RNasin, 0·5 U/μl	0	0
EDN alone	41	33
EDN + RNasin, 0·5 U/μl	29 (24)	27 (18)
EDN + yeast RNA, 4 mg/ml	0 (100)	0 (100)

* Monolayers of RHM were incubated with 1×10^{-4}M EDN at 37° for 6 hr; percentage lysis and percentage detachment were calculated as described in the Materials and Methods. This experiment was performed twice.

† %R, percentage reduction in RHM lysis or detachment with respect to the corresponding positive control value (EDN alone).

myofibres. In the myocardium of these patients we found very few eosinophils and could not detect deposits of EDN or EPO (Table 2).

Deposits of EDN or EPO were not detectable in sections from control heart tissue from patients who had died from a cerebral-vascular accident (normal heart tissue), acute myocardial infarction, myocardial infarction in the scarring stage and with arrhythmias, and from patients with myocarditis secondary to bacterial endocarditis (photos not shown). Eosinophils were only seen, and occasionally, in the heart tissue sections from the patients with acute myocardial infarction and bacterial endocarditis; the proportions of eosinophils in these cases were considerably smaller than those present in chagasic DN lesions (data not shown).

Toxic effects of EDN on myoblasts

Since several eosinophil granule components are toxic for mammalian cells *in vitro* (reviewed by Gleich & Adolphson, 1986), granule material secreted during eosinophil interaction with *T. cruzi* (Villalta, Pankratz & Kierszenbaum, 1987) might damage host tissues. Incubation of ^{51}Cr-labelled RHM with EDN resulted in cell lysis, evidenced by the significant release of radioactivity into the supernatant (Table 3). Furthermore, radioactivity contained in cells caused to become detached from the monolayer by EDN represented a considerable proportion of the total radioactivity of the culture. Damage caused to the RHM by EDN was also observed microscopically. In a typical experiment, the numbers of RHM per 100 fields in cultures incubated with medium alone, 1×10^{-5}M EDN and 1×10^{-4}M EDN, were 1907 ± 133, 1590 ± 71 and 286 ± 13, respectively. Although the cell loss produced by 1×10^{-5}M EDN was relatively small, most of the cells that remained attached to glass showed marked alterations, including a pyknotic, fragmented nucleus and shrunken cytoplasm.

The EDN has been ascribed ribonuclease activity (Slifman *et al.*, 1986) and this property appears to be involved in the destruction of *T. cruzi* trypomastigotes *in vitro* (H. A. Molina, F. Kierszenbaum, K. Hamann and G. J. Gleich, unpublished results). To find out whether this enzymatic activity was involved in the cell damage inflicted by EDN, we incorporated into the assay system yeast RNA as a competitive substrate or the ribonuclease inhibitor Rnasin. The former completely abrogated the toxic effect of EDN and the latter caused partial, though significant and reproducible inhibition (Table 3). Higher doses of RNasin, being toxic themselves, could not be tested in our assay.

DISCUSSION

Two observations implicated eosinophils in the development or aggravation of the most severe (degenerative, necrotic) type of myocardial lesion that frequently occurs in patients with Chagas' disease: a marked degree of eosinophil infiltration and the presence of deposits of eosinophil secretion products. This newly uncovered correlation has gone unnoticed in the past, probably because histological studies have emphasized the presence or absence of inflammatory cell types rather than their relative distribution in chagasic tissue lesions. The possible participation of eosinophils in lesion production was suggested by the *in vitro* toxicity of EDN for myoblasts. Eosinophil degranulation would expose myocardial cells not only to EDN but to other eosinophil granule components whose toxicity for mammalian cells has been recognized (Gleich *et al.*, 1979; Tai *et al.*, 1982; Young *et al.*, 1986). Eosinophil infiltration comparable to that of DN chagasic lesions was not present in heart tissues from patients dying from a cerebral-vascular accident (normal heart) or from heart diseases other than Chagas' disease. This difference raises intriguing questions about the cause of eosinophil accumulation in chagasic DN lesions that can not be answered with the present data.

Eosinophils were systematically present in DN and MM areas, although in variable numbers (cf. Tables 1 and 2). However, two features distinguished the DN from the MM histological pattern. First, the eosinophil infiltration was systematically more pronounced in the DN areas. Second, the number of eosinophils in DN but not in MM areas correlated

well with the overall degree of myocarditis evaluated histopathologically. Eosinophil infiltration represented, at least in our study case population, a measure of lesion severity. Our results, however, do not define what factors determine eosinophil accumulation in DN areas. Conceivably, these cells could be initially recruited to clear parasites released from bursting infected host cells and tissue debris and, upon degranulation, contribute to further tissue destruction. The smaller numbers of eosinophils found in MM and IF lesions might represent, with respect to the presence of necrosis and tissue degeneration, an earlier and later phase, respectively; a hypothesis deserving further study.

Only three of the patients showed the CL pattern in their myocardium. Eosinophil infiltration in CL lesions was erratic and deposits of EDN or EPO were not found, precluding any firm conclusion.

That the deposition of eosinophil secretion products also correlated with the severity of myocardial tissue damage was suggested by the presence of EDN and EPO deposits almost exclusively in DN areas, where these granule components were readily detectable both on the myofibres and in the interstitium (Table 1, Fig. 2). Furthermore, such deposits were virtually absent in the milder MM areas (Table 2).

Although eosinophil degranulation occurs *in vivo*, the levels of eosinophil granule components in infiltrated tissues are not known. Thus, we can not be certain that the concentrations of the toxic eosinophil secretion products, e.g. EDN, MBP, ECP (reviewed by Gleich & Adolphson, 1986), found to be toxic for mammalian cells *in vitro* (Gleich *et al.*, 1979; Tai *et al.*, 1982; Young *et al.*, 1986) are present at inflammatory lesion sites. However, we have observed that whole granules released by eosinophils after interaction with *T. cruzi* amastigotes become attached to bystander myoblasts (H. A. Molina and F. Kierszenbaum, unpublished observation) and also to non-internalized parasites (Villalta *et al.*, 1987). This direct attachment would expose the cells to essentially undiluted toxic granule components, i.e. to concentrations that could equate and possibly exceed the levels found to be toxic *in vitro*.

Degranulation, a process that generally accompanies eosinophil activation, occurs after eosinophil interaction with amastigote forms of *T. cruzi in vitro* (Villalta & Kierszenbaum, 1984a; Kierszenbaum *et al.*, 1986; Villalta *et al.*, 1987). Possibly, parasites released from infected host cells and/or host cell debris could induce a similar effect *in vivo*. The polycationic nature of MBP and ECP has been implicated in their ablities to lyse nucleated cells (Kierszenbaum, Ackerman & Gleich, 1981; Villalta & Kierszenbaum, 1984a; H. A. Molina, F. Kierszenbaum, K. Hamann and G. J. Gleich, unpublished results). Inhibition of the toxic effect of EDN on RHM by either yeast RNA or RNasin indicated that the RNase activity of this cationic protein was involved. It is tempting to speculate that these granule components, EPO—which can mediate cell lysis through the production of reactive oxygen metabolites (Jong & Klebanoff, 1980)—and secreted hydrolytic enzymes, could contribute to the destruction of heart myofibres in Chagas' disease. Such a process would not preclude concomitant damage by other mechanisms of pathogenesis that have been postulated to date (reviewed by Andrade & Andrade, 1979).

A possible role for eosinophils in the pathogenesis of myocardial chagasic lesions has not been suggested previously, possibly because, until very recently (Molina & Kierszenbaum, 1987), there had not been studies that exposed the correlation between the numeric accumulation of these cells and necrosis. This relationship may have gone inadverted before because eosinophils are not the most abundant inflammatory cell type in the myocardial infiltrates of chagasic patients. However, a role for eosinophils in the development of endocardial fibrosis has been postulated based on experimental as well as clinical evidence (Spry *et al.*, 1983; Spry *et al.*, 1985; Tai *et al.* 1987).

ACKNOWLEDGMENTS

We would like to thank Mr M. Schultz, Mr L. Masciotra and Mr L. R. S. Andrade for their assistance in the preparation of tissue sections. This work was supported by a Biomedical Research Support Grant from the College of Osteopathic Medicine of Michigan State University.

REFERENCES

ACKERMAN S.J., LOEGERING D.A., VENGE P., OLSSON I., HARLEY J.B., FAUCI A.S. & GLEICH G.J. (1983) Distinctive cationic proteins of the human eosinophil granule: major basic protein, eosinophil cationic protein and eosinophil-derived neurotoxin. *J. Immunol.* **131,** 2977.

ANDRADE Z.A. & ANDRADE S.G. (1979) Patologia. In: *Trypanosoma cruzi e Doença de Chagas* (eds Z. Brener and Z. A. Andrade), p. 199. Guanabara Koogan, Rio de Janeiro.

ANDRADE Z.A. (1985) A patologia da doença de Chagas no homem. *Ann. Soc. Belge. Méd. Trop.* **65**(Suppl. 1), 15.

GLEICH G.J. & ADOLPHSON C.R. (1986) The eosinophilic leukocyte: structure and function. *Adv. Immunol.* **39,** 177.

GLEICH G.J., FRIGAS E., LOEGERING D.A., WASSOM D.L. & STEINMULLER D. (1979) Cytotoxic properties of the eosinophil major basic protein. *J. Immunol.* **123,** 2925.

GLEICH G.J., LOEGERING D.A., BELL M.P., CHECKEL J.L., ACKERMAN S.J. & MCKEAN D.A. (1986) Biochemical and functional similarities between human eosinophil-derived neurotoxin and eosinophil cationic protein: homology with ribonucleases. *Proc. natl. Acad. Sci. U.S.A.* **83,** 3146.

JONG S. & KLEBANOFF S.J. (1980) Eosinophil-mediated mammalian tumor cell cytotoxicity: role of the peroxidase system. *J. Immunol.* **124,** 1949.

KIERSZENBAUM F., ACKERMAN S.J. & GLEICH G.J. (1981) Destruction of bloodstream forms of *Trypanosoma cruzi* by eosinophil granule major basic protein. *Am. J. Trop. Med. Hyg.* **30,** 775.

KIERSZENBAUM F., VILLALTA F. & TAI P.-C. (1986) Role of inflammatory cells in Chagas' disease. III. Kinetics of human eosinophil activation upon interaction with parasites (*Trypanosoma cruzi*). *J. Immunol.* **136,** 662.

KÖBERLE F. (1968) Chagas' disease and Chagas' syndromes: the pathology of American trypanosomiasis. *Adv. Parasitol.* **6,** 63.

LIMA, M.F. & KIERSZENBAUM F. (1982) Biochemical requirements for intracellular invasion by *Trypanosoma cruzi*: protein synthesis. *J. Protozool.* **29,** 566.

MOLINA H.A. & KIERSZENBAUM F. (1987) A study of human myocardial tissue in Chagas' disease: distribution and frequency of inflammatory cell types. *Int. J. Parasitol.* **17,** 297.

ROMAÑA C. (1963) Anatomía patológica, patogenia e immunología. In: *Enfermedad de Chagas*, p. 97. Lopez Libreros, Buenos Aires.

ROWLAND E.C. & SIBLEY-PHILLIPS S. (1984) Bone marrow eosinophil levels in *Trypanosoma cruzi*-infected mice. *J. Parasitol.* **70,** 819.

SCHLESINGER M.J. & REINER L. (1955) Focal myocytolysis of the heart. *Am. J. Pathol.* **31,** 443.

SLIFMAN N.R., LOEGERING D.A., MCKEAN D.J. & GLEICH G.J. (1986) Ribonuclease activity associated with human eosinophil-derived neurotoxin and eosinophil cationic protein. *J. Immunol.* **137,** 2913.

SPRY C.J.F., DAVIES J., TAI P.-C., OLSEN E.G., OAKLEY C.M. & GOODWIN J.F. (1983) Clinical features of fifteen patients with hypereosinophilic syndrome. *Quart. J. Med.* **52,** 1.

SPRY C.J.F., WEETMAN A.P., OLSSON I., TAI P.-C. & OLSEN E.G. (1985) The pathogenesis of eosinophilic endomyocardial disease in patients with carcinoma of the lung. *Heart Vessels*, **1**, 62.

TAI P.-C., HAYES D.J., CLARK J.B. & SPRY C.J.F. (1982) Toxic effects of eosinophil secretion products on isolated rat heart cells *in vitro*. *Biochem. J.* **204**, 75.

TAI P.-C, SPRY C.J.F., OLSEN E.G.J., ACKERMAN S.J., DUNNETTE S. & GLEICH G.J. (1987) Deposits of eosinophil granule proteins in cardiac tissues of patients with eosinophilic endomyocardial disease. *Lancet*, **i**, 643.

VILLALTA F. & KIERSZENBAUM F. (1983) Role of polymorphonuclear cells in Chagas' disease. I. Uptake and mechanisms of destruction of intracellular (amastigote) forms of *Trypanosoma cruzi* by human neutrophils. *J. Immunol.* **131**, 1504.

VILLALTA F. & KIERSZENBAUM F. (1984a) Role of inflammatory cells in Chagas' disease. I. Uptake and mechanism of destruction of intracellular (amastigote) forms of *Trypanosoma cruzi* by human eosinophils. *J. Immunol.* **132**, 2053.

VILLALTA F. & KIERSZENBAUM F. (1984b) Role of inflammatory cells in Chagas' disease. II. Interaction of mouse macrophages and human monocytes with intracellular forms of *Trypanosoma cruzi*: uptake and mechanisms of destruction. *J. Immunol.* **133**, 3338.

VILLALTA F., PANKRATZ H.S. & KIERSZENBAUM F. (1987) Extracellular killing of *Trypanosoma cruzi* amastigotes by human eosinophils. *J. Protozool.* **34**, 285.

YOUNG J.D., PETERSON C.G., VENGE P. & COHN Z.A. (1986) Mechanism of membrane damage mediated by a human eosinophil cationic protein. *Nature (Lond.)*, **321**, 613.