

Removal of *Trypanosoma cruzi* by white cell-reduction filters: an electronmicroscopic study

Remoção de *Trypanosoma cruzi* através de filtros para redução de leucócitos: um estudo eletromicroscópico

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Abstract White cell (WBC)-reduction filters have been shown to be effective in removing infectious agents from infected blood products. In this study, the mechanisms of *Trypanosoma cruzi* (*T. cruzi*) retention by WBC-reduction filters were assessed. Human packed red blood cell (PRBC) and platelet concentrate (PC) samples were contaminated with *T. cruzi* organisms (Y strain; $3.4 \times 10^6/\text{ml}$), and then filtered using WBC-reduction experimental filters that provided about $3 \log_{10}$ WBC removal. Transmission electron microscopy sections showed that *T. cruzi* parasites were removed from contaminated PRBC and PC samples primarily by mechanical mechanism without interacting with filter fibbers or blood cells. In addition, we found that *T. cruzi* parasites were also removed by a direct fibber adhesion. These data indicate that *T. cruzi* parasites are removed from infected blood not only by mechanical mechanism but also by biological mechanism probably mediated by parasite surface proteins.

Key-words: Chagas' disease. White cell-reduction filter. Blood filtration. Electron microscopy.

Resumo Os filtros de leucócitos são eficazes em remover agentes infecciosos de hemocomponentes infectados. Neste estudo, foram avaliados os mecanismos de retenção de *Trypanosoma cruzi* (*T. cruzi*) por filtros de leucócitos. Amostras de concentrado de glóbulos vermelhos (CGV) e concentrado de plaquetas (CP) foram contaminados com parasitas *T. cruzi* (cepa Y, $3,4 \times 10^6/\text{ml}$), e filtrados com filtros experimentais de leucócitos, com capacidade de remoção de aproximadamente $3 \log_{10}$ leucócitos. A análise das fibras dos filtros através de microscopia eletrônica de transmissão mostrou que os parasitas *T. cruzi* foram removidos dos CGV e CP contaminados por mecanismo físico, sem interação com células sanguíneas. Além disso, foi demonstrado que os parasitas *T. cruzi* foram também removidos por adesão direta às fibras dos filtros, sugerindo um mecanismo biológico, provavelmente mediado por proteínas da superfície do parasita.

Palavras-chaves: Doença de Chagas. Filtros de leucócitos. Filtração de sangue. Microscopia eletrônica.

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Chagas' disease is a zoonosis caused by flagellate protozoan parasite *Trypanosoma cruzi*⁸. The disease is widespread in Latin America where about 18 million people were infected and 90 million people were at risk in the early 90's^{11 18 20}. With the eradication, in some countries, of the blood-sucking triatome insects which transmit *T. cruzi*, allogeneic blood transfusions have been the main route of transmission of Chagas' disease in urban areas of both endemic and non-endemic countries^{11 18}. Over the past years, the problem was limited to Latin America however, with the continuous emigration of *T. cruzi*-infected people to developed countries and the report of 4 cases of transfusion-associated Chagas disease (TA-CD) in immunocompromised patients^{10 11 15 18}, the risk of *T. cruzi* transmission by blood

filters from contaminated blood products.

MATERIAL AND METHODS

Blood components. Trypomastigote forms of *T. cruzi* (Y strain) were obtained from infected blood of male swiss mice, which are routinely maintained in our laboratory. Human whole blood was collected in triple bags with CPDA-1 (Baxter Healthcare Corporation, Fewal Division, Mexico). After collection, whole blood units were immediately centrifuged for 4 minutes (1,800g, 22°C) and the platelet-rich plasma was transferred to the satellite bag using the heat-sealing method. The packed red blood cell (PRBC) concentrate was kept at room temperature for about 2 hours. After this short time storage, PRBC samples of 5ml were contaminated with 17×10^6 *T. cruzi* (3.4×10^6 per ml). The parasites were mixed with red cells from PRBC by tube inversion (5x) and kept at room temperature for an extra hour, before filtration. In order to prepare platelet concentrates (PCs), platelet-rich plasma unit was centrifuged for 10 minutes (3,560g, 22°C) and the platelet-poor plasma unit was transferred to the satellite bag using the heat-sealing method. A PC sample was kept at room temperature (22°C), under orbital agitation. After a four-day storage period, PC samples (5ml) were contaminated with 17×10^6 *T. cruzi* (3.4×10^6 per ml) and kept at room temperature for one hour, before filtration.

Enumeration of the parasites. The enumeration of *T. cruzi* parasites from contaminated mice whole blood from human PRBC and PC samples was performed before and after filtration by

transfusions is increasing also in non-endemic countries. Currently, the strategies to prevent TA-CD include general approaches such as education and identification of putatively infectious blood donors by questionnaire; serologic tests; and in areas of high endemicity, the treatment of the collected blood with gentian violet^{1 7 14 17}.

Based on the evidence that white cell (WBC)-reduction filters are effective in preventing of some biologic effects caused by allogeneic blood transfusions^{4 12}; in removing virus and bacteria present in blood components^{4 5 9}; and, in directly removing *T. cruzi* from contaminated blood¹³, the present study investigated, by electron microscopy, the possible mechanisms involved in the removal of *T. cruzi* by WBC-reduction

Brener's method⁶. Briefly, 5µl of contaminated samples from either mice whole blood or human blood, were spread in a slide with a 22 x 22mm cover-slip to form a single layer of blood cells. The number of trypanosomes present in the samples was investigated by scoring the parasites observed into 50 fields and by multiplying the score by 52. The constant used in this calculation was previously estimated under a 40x objective and a 10x ocular⁶.

Blood component filtration. The experimental WBC-reduction filters used in this study were made of cellulose acetate fiber with 5ml capacity and provide about 3 log₁₀ leukocyte removal (Miles Inc., Berkeley, CA). This filter efficacy has been previously demonstrated in experimental animal models^{2 3}. The filtration of five samples of artificially *T. cruzi*-infected PRBC was performed by diluting the PRBC to achieve a haematocrit of 30 percent (0,30) with saline. While the filtration of four samples of PC was performed directly, without diluting. The filtration through the WBC-depletion filter was made using a 5ml syringe. The procedure took about 1 minute.

Electron microscopic examination. Immediately after filtration, the WBC-reduction filters were opened and embedded with 2-percent glutaraldehyde in 0.1M sodium cacodylate for electron microscopic examination. After 1 minute in this solution, the filters were closed and kept for at least 24 hours at 4°C. To examination by transmission electron microscopy (TEM), centre and peripheral samples of the filters were selected. We rinsed the samples in 0.1M sodium

cacodylate for twice, each (15 minutes) and then kept for 12 hours at room temperature. After the incubation, the fibers were fixed with 0.1M osmium tetroxide for 60 minutes at room temperature. The samples were rinsed again, and dehydrated in graded alcohols (50-100%) for 30 minutes, and in propylene oxide for 15 minutes. Subsequently, the samples were infiltrated with

then examined in a transmission electron microscope (JEOL, JEM-1200 EX II, Japan).

RESULTS

Parasite removal. Confirming the observations reported previously¹³, the results of the present study showed that the WBC-reduction experimental filters were partially effective in

removing *T. cruzi* not only from contaminated PRBC but also from infected PC. The filtration of the contaminated PRBC samples was 40% effective, resulting in a parasite count reduction from 3.4 to 2 x 10⁶/ml. The filtration of the contaminated PCs samples was 53% effective,

removing *T. cruzi* not only from contaminated PRBC but also from infected PC. The filtration of the contaminated PRBC samples was 40% effective, resulting in a parasite count reduction from 3.4 to 2 x 10⁶/ml. The filtration of the contaminated PCs samples was 53% effective,

Tabela 1 - Number of *T. cruzi* parasites from contaminated human packed red blood cell and platelet concentrate before and after filtration and the percent of parasites removed by WBC-reduction filters.

Blood component	Number of parasites		Retention (%)
	pré	pós	
PRBC	17 x 10 ⁶	10 x 10 ⁶	40
PC	17 x 10 ⁶	8 x 10 ⁶	53

resulting in a parasite count reduction from 3.4 to 1.6 x 10⁶/ml (Tabela 1).

Microscopic studies by TEM sections

showed that *T. cruzi* parasites were removed from contaminated PRBC and PCs primarily by mechanical mechanism with parasites not



Figure 1 - Photomicrograph obtained after filtration of *T. cruzi*-contaminated PRBC by a WBC-reduction filter. Transmission electron microscopy showing fibers (F) in cross-section, red cells (RBC), and a trypomastigote form of *T. cruzi* (T). Magnification x 11.500.

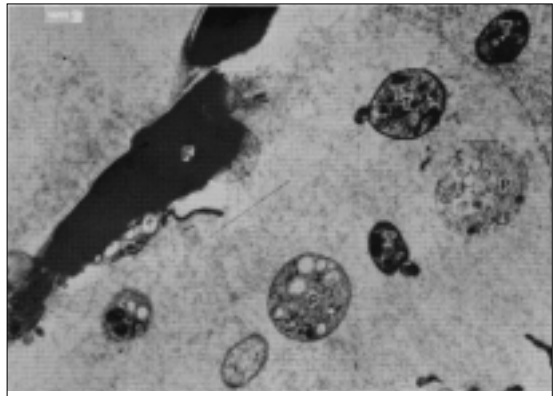


Figure 2 - Photomicrograph obtained after filtration of *T. cruzi*-contaminated PC by a WBC-reduction filter. Transmission electron microscopy showing a fiber in cross-section (F), platelets (P), and trypomastigote forms of *T. cruzi* (T). Magnification x 11.500.

interacting neither with fibers nor with blood cells (Figures 1 and 2). However, in an additional

experiment showed that a *T. cruzi* parasite from contaminated PRBC was removed by a direct

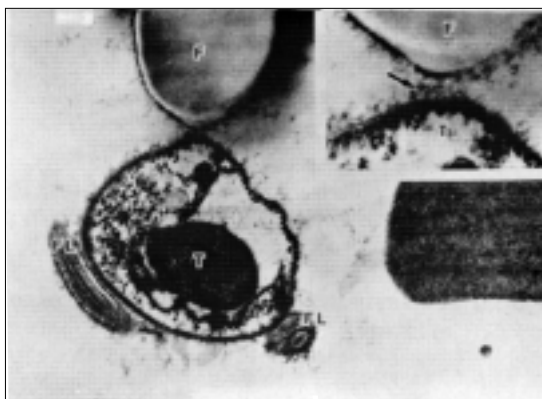


Figure 3 - Photomicrograph obtained after filtration of *T. cruzi*-contaminated PRBC by a WBC-reduction filter. Transmission electron microscopy showing a fiber (F) in cross section, and a trypomastigote form of *T. cruzi* (T) with a visible flagellum (FL). Magnification x 33.000. The upper right corner shows the fiber (F) in close contact (arrow) with the trypomastigote form of *T. cruzi* (T). Magnification x 84.000.

fiber adhesion (Figure 3), characterizing a biological mechanism of retention (parasite-fiber).

DISCUSSION

It has been recently suggested that WBC-reduction filters may prevent the transmission of virus and bacteria present in blood components⁹. The retention of infectious agents by WBC-reduction filters is ordinarily determined by the type of virus and therewith the binding towards WBCs; the type of bacteria and holding period before filtration; the deformability of infected cells; and the desintegration of cells in the filter¹⁹. Two mechanisms have been proposed to explain reduced bacterial growth in WBC-reduced blood products: removal of WBCs that have phagocytosed bacteria and direct removal of bacteria by the filter itself. However, neither mechanism has been distinguished directly by studies determining bacterial localization perfiltration⁹.

Recently, we have reported the efficacy of various WBC-reduction filters in directly removing *T. cruzi* from contaminated blood¹³. To extend our previous observations, in the present study we evaluated, by electron microscopy, the possible mechanisms involved in the removal of *T. cruzi* from artificially contaminated blood components

samples. Using the scoring method, it was possible to confirm that WBC-reduction experimental filters are partially effective in removing *T. cruzi* from contaminated blood components. In addition, using TEM we found that *T. cruzi* was removed from contaminated PRBC by both mechanical (barrier retention), and biological mechanisms. Probably, the fiber adhesion occurs due to surface characteristics of parasite *T. cruzi*. It is possible that glycoproteins present in the surface of parasite participate in the direct adhesion of the parasites to the fibbers of the filters. In this context, it was recently identified a protein with 60Kd (penetrin) at the surface of *T. cruzi* parasites that mediates the adhesion of the organisms to components of extra-cellular matrix, as collagen¹⁶. In the present study, we also demonstrated that *T. cruzi* parasites were removed from infected PCs by WBC-reduction filters without interacting with filter fibbers or blood cells, suggesting a mechanical mechanism of retention.

In conclusion, the present data confirm that WBC-reduction filters are capable of removing *T. cruzi* parasites from contaminated blood components; and that the parasites retention

occurred mainly by mechanical but also by biological mechanisms. However, clinical studies with *T. cruzi*-infected persons and using WBC-

transmission of parasites by allogeneic blood transfusions.

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reduction filters of better capacity of leukocyte reduction are necessary to ascertain the clinical efficacy of WBC-reduction filters in reducing the

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