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# PROTECTIVE ANTIBODIES IN *TRYPANOSOMA CRUZI* INFECTIONS: DETECTION, FUNCTIONAL ACTIVITY AND POSSIBLE MECHANISMS OF TRYPOMASTIGOTE KILLING *IN VIVO* AND *IN VITRO*

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During the chronic phase of *Trypanosoma cruzi* infections in humans and in experimental hosts there is a strong specific humoral immune response mediated by different types of immunoglobulins (review: Krettli, 1982, 1983). They are very useful for diagnosis of this phase since parasitemia is subpatent. Several serological methods have been employed, both for individual and epidemiological diagnosis. As we previously showed, only some of the serum antibodies are protective against and otherwise lethal dose of *T. cruzi* (Krettli & Brener, 1982). Furthermore, a strain-dependent sensitivity towards antibody protection is well known (Krettli & Brener, 1976). Thus, using as a target cell a highly sensitive *T. cruzi* strain (Y) we have been studying: (a) possible methods to identify protective antibodies *in vitro*, and *in vivo*; (b) mechanisms of parasite killing by antibodies *in vitro* and *in vivo*; (c) their distinction from other immunoglobulins specific against *T. cruzi* but which are not protective. So far, any method in which the target cells are the living trypomastigotes instead of the traditional methods using fixed epimastigotes, detects protective antibodies and discriminate them from other non-protective immunoglobulins. Since the complement-mediated lysis test (CoML) has been preferably used in our studies, we often refer to protective antibodies as "lytic antibodies" to differentiate them from antibodies involved in conventional serology (CS). The latter are also present in immunized hosts (Krettli & Brener, 1982) and in some patients specifically treated (Krettli, Cançado & Brener, 1982) but they do not recognize living trypomastigotes neither in the CoML nor in the other methods herein described.

## Methods to detect protective antibodies *in vitro*

The distinction between protective and non-protective antibodies was first demonstrated in our recent work showing that only the former will bind to living trypomastigotes and convert those cells into activators of the complement system thus being lysed by fresh serum. Conversely, sera from non-infected but *T. cruzi* immunized mice (not significantly protected) were shown to be unable to bind to the living trypomastigotes, therefore would not induce such *in vitro* lysis (Krettli & Brener, 1982). The idea that "lytic antibodies" are the same protective antibodies has been further corroborated by several groups as discussed further in. Other methods using the living trypomastigotes also allow the detection of protective antibodies such as immunofluorescence tests, antibody-dependent cell cytotoxicity, phagocytosis by macrophages, agglutination tests and serum neutralizing activity.

a) **CoML**: the trypomastigotes (Try) with no membrane-bound immunoglobulins needed for this test are obtained from acutely infected X-irradiated or drug immunosuppressed mice (cyclophosphamide); from cell cultures; and from acellular liquid cultures (LIT or other media). The details for the purification of the blood parasites as well as for the CoML have been described (Krettli, 1978; Krettli, Weisz-Carrington & Nussenzweig, 1979). Briefly, a purified suspension of Try diluted with protein enriched medium (5% of foetal calf serum – FCS, or 1% of crystalized bovine serum albumine – BSA) is incubated with the diluted test sera for 30 min at 37°C and aliquots are then incubated 1h 37°C with fresh human serum (HuS) as a source of complement and with heat inactivated serum (56°C 30 min) as control (iHuS). More recently we have modified this technique as follows: (i) the first step of incubation i.e., serum plus Try is performed at 4°C in the presence of 0.05% $NaN_3$ (sodium azide) to avoid capping and shedding of the membrane-bound immunoglobulin plus the antigen specific epitopes. In the absence of azide capping is intense and rather rapid even at 4°C (Schmunis et al., 1978; Katzin et al., 1984); (ii) the control tubes are Try plus normal serum and complement. This modification aimed to eliminate the background error caused by the strong agglutination of the Try with iHuS plus sera from chronic infections. Another way to eliminate the background error caused by agglutination is to count the Try suspension before and after incubation with HuS as performed by Kipnis et al. (1981).

The CoML is a direct and simple test which was first reported in the literature by Kierszenbaum (1976) using sera from chronic infections and Try from different strains as target cells. He found that serum from the chronic phase induced lysis of the various parasites. Independently, in an attempt to characterize the anti-*T. cruzi* humoral response, we were using the CoML and immunofluorescence tests to detect membrane-bound immunoglobulins on the surface of *T. cruzi in vivo* (Krettli, 1978). We found that circulating Try from CL and Y strains isolated from immunocompetent mice had bound immunoglobulins on their membrane, a phenomenon also demonstrated at the time by Kloetzel & Deane (1977) using the immunofluorescence technique. However, we observed that the immunoglobulins circulating in the acute phase

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differed from the protective antibodies previously described in the chronic phase. Only the later displayed a cross-reacting ability to mediate the complement-lysis of bloodstream trypomastigotes, regardless of the strain used as a target cell (Kierszenbaum, 1976; Krettli & Nussenzweig, 1977; Krettli, 1978; Krettli, Weisz-Carrington & Nussenzweig, 1979). However, the *in vivo* induction of lytic antibodies in the acute phase was shown to be strain-independent (Krettli & Nussenzweig, 1977). This was not the case of the target cell used for lysis and upon incubation with serum from the acute phase. Only the Y and Berenice strains are agglutinated and/or lysed, whereas strains like CL, Gilmar and others are resistant to lysis and agglutination by this sera. In conclusion, there is a strain cross-reactivity of lytic antibodies from the chronic phase but not from the acute phase. Another important difference between anti-*T. cruzi* sera from the acute and chronic phases is that only the former is protective although both have lytic activity. Differences in titers, further discussed in details as well as differences in isotypes may help to discriminate both types of immunoglobulins detected by the CoML and clarify the lack of protective activity in serum from early infections.

There are problems which limit the CoML test, for instance the anticomplementary effect of some human sera as we discussed in another paper in this journal. Although the CoML is the current method to demonstrate protective antibodies, its limits, besides of course the danger of manipulating living *T. cruzi*, are: the need of a continuous source of living Try; the use of irradiation machines (both X-rays and $\gamma$-rays can be used at the dose of 550-650 rads); or, alternatively, of a continuous cell-culture system; human fresh sera previously assayed as a complement source and kept at -70°C; the need of skilled and endured personal to count the fast moving creatures with a Neubauer chamber!

b) **Direct agglutination**: although this has been the first method we used to detect protective antibodies using as a target cell the Y-trypomastigotes from mouse blood (Krettli & Brener, 1976) we have abandoned the technique because the agglutination may be induced also by heterophilic antibodies from non-chagasic humans. In this case, the non-specific reaction may be eliminated by previous absorption of the sera with mouse erythrocytes (Krettli, 1978, 1979). The use of diluted serum (1:8, 1:16) increases the specificity of the method however it decreases its sensitivity. Anyway, this may represent an alternative technique for epidemiological purposes in which a very large number of positive sera may be screened. Later tests by CoML or another method specific for *T. cruzi* protective antibodies will further indicate ongoing infections.

c) **Indirect immunofluorescence with living trypomastigotes (IIF-L)**: the technique previously described by Krettli & Brener (1982) consists in incubating Try plus diluted serum for 30 min at 37°C followed by 3 washes with phosphate buffered saline (PBS 0.15 M pH 7.2) containing 0.2% $NaN_3$ (azide) and 5% FCS. The parasites are then incubated 3 h at 4°C with a fluorescein-labelled anti-immunoglobulin (anti-mouse or -human IgG) washed twice as above and examined under light microscope and UV light (Ortholux Leitz microscope). The Try in the presence of azide has the double advantage of inhibiting shedding and of slowing down the parasite movements, which, even thus, make the direct reading of the fresh preparations rather difficult. Furthermore, the control Try in the presence of normal serum may display a background fluorescence represented by small dots on their surface. There is a strong difference between such background fluorescence and a positive reaction, given by the projective antibodies in which most but not all Try show a strong diffuse membrane fluorescence. The IIF-L technique has been largely improved by the modification introduced by Umezawa et al. (1983) and briefly it consists of fixing the trypomastigotes, after their incubation with diluted serum and washing, with 2% formaldehyde for 2 h at 37°C, washing again and preparing air dried slides which are stained with fluoresceine-conjugated anti-serum. The authors detected a dotted fluorescence mainly on the extremities of the trypomastigotes, in the positive tests. We have also used the modified test and found it easier to read but the fixation step is at 4°C with para-formaldehyde. We found that sera from chronic infections displaying protective antibodies always showed an intense and uniforme fluorescence of most Try. Serum from immunized non-infected mice as well as normal serum, may also induce a dotted fluorescence, very distinct, however, from the positive serum.

d) **Antibody-dependent cytotoxicity (ADCC)**: the ADCC with trypomastigotes from immuno-suppressed mice and non-adherent normal mouse spleen cells is able to identify protective antibodies and to discriminate them from non-protective antibodies involved in conventional serology (Lima-Martins et al., 1983; Lima-Martins, 1984; Lima-Martins; Krettli & Brener, 1985). Cytotoxicity is expressed as the percent reduction in the number of trypomastigotes after 5 h at 37°C compared to the number of parasites in the presence of normal cells and serum. Sera from chronically infected mice and untreated humans always mediated ADCC killing of the trypomastigotes. Conversely, sera from mice immunized with killed *T. cruzi* or from some treated chagasic patients (with negative CoML but positive CS) mostly presented negative ADCC as did normal sera from mice or humans. Trypsinization of the trypomastigotes increases $\geqslant$ 100 fold the ADCC lysis. However, in this case both antibodies involved in CoML and in CS mediated the parasite ADCC lysis (Lima-Martins, 1984). Therefore the ability of the trypomastigotes to discriminate between the protective antibodies depend on the presence of an intact surface coat synthesized by the parasite. The participation of serum-proteins adsorbed on the parasite surface membrane in this discriminative capacity of the living trypomastigotes towards some functional immunoglobulins may also occur but has not been yet investigated. Finally, the isotypes mediating ADCC are IgG, mainly $IgG_{2a,b}$.

e) **Phagocytosis of living trypomastigotes**: similarly to the above results with ADCC, it has been shown that only sera from active chronic infections (humans, mice, rabbits) display a significant ability to

opsonize trypomastigotes thus enhancing phagocytosis by normal peritoneal macrophages from mice. Sera from immunized non-infected animals or from some treated patients with negative CoML but high titers of serological antibodies (1:640) had an opsonizing effect on *T. cruzi* trypomastigotes similar to normal sera (Lages Silva, Krettli & Brener, 1983).

f) **Serum neutralizing activity (SNA)**: the antibodies directed against epitopes on the surface membrane of living trypomastigotes (ALBA) *in vitro* are able to decrease their virulence *in vivo* (Krettli & Brener, 1976). The technique is simple and very useful and consists in incubating trypomastigotes with sub-agglutinating dilutions of ALBA positive sera at 28-33°C 1h or at 4°C $\geqslant$ 2h and inoculation of mice ($10^3$-$10^4$ parasites/An.). Parasitemia and mortality at the acute phase in mice infected with this suspension when compared to groups receiving normal serum-treated *T. cruzi,* is significantly reduced. The SNA has also been used (Gonzales-Cappa et al., 1979, 1980; Sanchez & Gonzalez-Cappa, 1983) to provide evidences that only one in 6 *T. cruzi* strains studied (CA1) was unable to induce the production of SNA antibodies during the chronic phase. Recently, we have been trying to clarify further whether there is a strain-specificity in the production of protective antibodies using individual mouse sera from 3-11 months chronic infections. Eleven different strains (Y, FL, Ernane, Gilmar, J, Generoso, JAT, SMJ, DM, JM and 7557) isolated from human cases; and one (CL) from a triatoma-bug were used in the SNA, CoML and CS. The Y strain was always used as a target cell. Both CoML and CS were positive in all of the 38 sera tested at high titers (up to 1:320). Twenty individual sera randomly tested by SNA were mostly protective (18 sera = 90%) and presented either a strong activity against *T. cruzi* (60% of sera) or mild activity (40% of sera). Only 2 sera (anti-JAT; anti-JM) had no SNA activity when tested at a 10-fold dilution although other mice from the same groups displayed SNA (Krettli et al., 1984). We conclude that the production of protective antibodies in mice is strain-independent and apparently the host individual to immune response rarely influences SNA induction. However, it may be that the methods and strains used to induce a chronic phase and the time elapsed between inoculation may interfere with the production of SNA, as well as of protective antibodies. For instance, a lack of protective immunity was found in mice chronically infected with the F strain and challenged with the Y strain although the animals had been protected against the homologous challenge (Kloetzel & Lafaille, 1983).

**Passive transfer of protection by sera and relevant antigens**

Until a few years ago there was an apparent controversy in the literature towards the ability of specific immune sera to protect naive hosts against *T. cruzi*. A significant protection described by some authors would be a complete failure in other similar attempts (review in Krettli, 1978; 1979; 1982). In 1976 we described the phenomenon of strain susceptibility towards the effects of anti-*T. cruzi* serum *in vitro* and *in vivo*. Populations predominantly slender (Y and Berenice strains) are strongly agglutinated and partially inactivated, both, after incubation *in vitro* with sera from chronic infections and after inoculation in naive hosts previously sensitized with these sera (Krettli & Brener, 1976; Krettli, 1979). Conversely, strains predominantly broad (CL, Gilmar, MR) were neither affected *in vitro* nor *in vivo* by those procedures. This finding was further confirmed by McHardy (1977). Therefore, by using the right target cell, proved to be antibody-sensitive, including the Tulahuen strain (Kagan & Norman, 1961; Hanson, 1977), a consistent and significant protection is achieved.

As discussed in the previous item, the protective antibodies are functional immunoglobulins directed against epitopes present on the membrane of living trypomastigotes as detected by CoML, IIF, SNA, ADCC and phagocytosis. They are located on the IgG isotype (Castelo Branco, 1978; Takehara et al., 1981) and either include IgG, $IgG_{2a,b}$ and probably $IgG_3$ (Stephani, Takehara & Mota, 1983; Romeiro, Takehara & Mota, 1984), or they are exclusively $IgG_{2a \text{ and } b}$ (Sanchez, Krettli & Brener, 1982; Lima-Martins, Krettli & Brener, 1985).

Most of the previous work on *T. cruzi* protective antibodies have been performed with sera from chronically infected mice inoculated with Try more than once. Recent results show that after about two months of infection the levels of protective antibodies, and, as expected of lytic antibodies, are very high and stable. They are not modified by further challenges (up to 4 re-inoculations) and/or by the time of infection (Pereira & Krettli, 1982; 1983). Protective antibodies have in general been associated to *T. cruzi* ongoing infections but are not induced by immunization with killed parasites or their fractions (Krettli & Brener, 1982). Mice immunized with the purified glycoprotein GP 72 have been used to select hybridomas from which one monoclonal had titers of $10^{-5}$ in CS against epimastigotes antigens. This highly specific monoclonal however had no protective effect against. *T. cruzi* (Snary et al., 1981). Recently, two groups have communicated their success on the production of lytic antibodies which were also protective antibodies after mice immunization with either parasite fractions or inactivated metacyclic trypomastigotes. In the first case (Campanini et al., 1984) obtained $IgG_1$ monoclonal antibodies directed against the flagellar fraction of *T. cruzi,* some of which recognize epimastigotes and trypomastigotes, have lytic activity detected by CoML and are partially protective against a parasite challenge. In the second case, Yoshida (1985) has been able to induce a strong non-sterile resistance immunizing mice with multiple doses of cultured-or insect-derived metacyclic trypomastigotes killed by heat (50°C 10 min) or by merthiolate treatment. Sera from these mice had a strong lytic activity and recognize surface proteins of molecular weight between 77-88 kd expressed on the membrane of living metacyclic trypomastigotes. Mice immunized with formaldehyde-treated trypomastigotes were not protected and their sera did not immunoprecipitate the above

mentioned glycoproteins, which are like to be the protective antigens. Therefore, Yoshida seems to be the first one who has induced a strong humoral immunity after immunization. Although rather unlike, the possibility of subpatent infections induced by the vaccination procedures have yet to be totally excluded in her studies. An example that this may occur is seen in the results of vaccination with trypomastigotes inactivated by 8-methoxy psoralen (MOP). The MOP-treated parasites, like the untreated ones, were invasive to cells however their capacity to replicate intracellularly was totally inhibited. When inoculated into mice they induced a strong antibody mediated protection with an apparent absence of infection (Andrews et al., 1985). The authors found later that at least some of their "vaccinated" mice were indeed chronically infected.

Trypomastigotes-specific monoclonal antibodies obtained from mice "vaccinated" with MOP-treated *T. cruzi* are capable to block *T. cruzi* cell invasion and recognize a GP-85 stage specific antigen (Alves, Abuin & Colli, 1984). Antibodies from chagasic patients sera also recognize the GP-85 and partly blocked the interiorization of *T. cruzi* in non-professional phagocytic cells (Zingales et al., 1982). Therefore the authors suggest that the GP-85 present only in trypomastigotes seems to be responsible for parasite interiorization.

In an attempt to discriminate between antigens recognized by protective antibodies and non-protective anti-*T. cruzi* immunoglobulins, Martins et al. (1984) have compared sera from humans or mice with a positive CS but in which CoML was positive or negative. In the case of humans, sera was obtained from well studied patients (5 years follow-up by our groups) specifically treated or not. The mice sera were from chronic infections or after immunization with killed epimastigotes. The authors found that one polipeptide of high molecular weight (160 kd) was recognized only by sera from humans or mice chronically infected by *T. cruzi* and therefore resistant to challenge infections. This polipeptide may well be a dimer of the 77-88 GP described in the above discussed works. The work now carried out must clarify the importance of this group of glycoproteins in the protective immunity.

The apparent difference between the successful immunization leading to protection presently discussed and previous results of weak or no protection after vaccination (reviewed by E. Camargo in another chapter) is the antigen used. The metacyclic or cultured trypomastigotes rather than epimastigotes or blood forms seem to be the right target and may express fully the relevant epitopes for the elicitation of an efficient protective immune response. Of course, we now seem to be closer to the isolation and identification of the antigen(s) which induce the protective (and lytic!) antibodies. The next step will be its use in simpler and easier to perform tests to detect CoML and to induce protection without the use of "inactivated" but entire parasites.

**Kinetics of appearance of protection and protective antibodies**

The kinetics of appearance of both, protective and CoML antibodies have been studied in groups of 50-60 mice inoculated with either Y or CL strain (1-5 x $10^4$ trypomastigotes per mouse i.p.) treated with a single subcurative dose of benznidazol (1.000 mg/kg p.o.). The mice were bled at approximately one week intervals for sera used in the detection of CoML and CS antibodies. They were then used for experiments of reinoculation of *T. cruzi* and for Try clearance to evalute the *in vivo* acquired resistance. Some of our findings, with the Y strain, are illustrated on Table I. As previously shown (Krettli, 1978; Krettli, Weisz-Carrington & Nussenzweig, 1979), lytic antibodies started to circulate at the first week after inoculation although their titers were very low up to the 3rd week. From the 4th week on, the titers were high (1:320) and remained steady onwards. Antibodies were detected by CS from the 3rd week in titers which gradually increased up to the 6th week.

The experiments of clearance were performed inoculating i.v. the normal control and test groups (3 mice each) with 2-3 x $10^6$ bloodstream Y-trypomastigotes isolated by differential centrifugations (Krettli, 1978). After different time, 5 $\mu$l blood samples from the inoculated mice tails were drawn to evaluate the number of circulating trypomastigotes (times 1, 3, 5, 10, 15, 20 min and at 10 min intervals up to 110 min). It has been previously shown that *T. cruzi* slender forms (like the Y and Berenice strains) circulate in the normal mouse during the first hour then gradually disappear initiating their intracellular cycle whereas in chronically infected mice the parasites are totally removed from circulating in 1 h (Brener, 1969). In our experiments from 12 to 27 days after prime inoculation the number of challenge trypomastigotes circulating was similar to the normal control groups during the first 90 min. Afterwards the number of parasites was slowly decreasing from the peripheric blood. About 40% of the parasites were still present after 160 min in both groups. In mice with 31-35 days of infection, although clearance was significantly faster than in the normal groups, about 50% of the inoculated trypomastigotes were still circulating after 90 min. However, from day 43 of infection onwards, 90 to 100% of the inoculated trypomastigotes were removed from circulation almost immediately after inoculation (3-10 min).

The appearance of lytic antibodies in high titers seemed to parallel the ability of the infected mice to remove the *T. cruzi* challenge (Table I). However, the acquired resistance against the parasite measured by parasitemia (fresh blood exam) and mortality was nill in all the chronically infected groups, from day 12 onwards. Therefore, there are apparently two distinct mechanisms of acquired immunity during *T. cruzi* infections, at least in mice in which the chronic phase is induced by chemotherapy after parasite inoculation. One, at the early phase in the first month of infection, which can not be transferred by sera (Krettli &

Brener, 1976; Krettli & Pereira, in preparation) neither detected by the test of clearance herein described. The second mechanism, most important at the late chronic phase of infection (from 1 month onwards) is mediated by protective antibodies. At this phase, the passive transfer of protection is easily achieved by sera. Furthermore, the reinoculation of viable trypomastigotes is immediately followed by parasite clearance from circulation and their killing. Whether this *in vivo* parasite antibody induced lysis is mediated by complement and/or ADCC and/or phagocytosis is still unclear and a matter to be clarified. Probably, several mechanisms collaborate for parasite killing at the chronic phase, thus explaining the strong acquired *T. cruzi* resistance displayed by the vertebrate infected host (review in Brener, 1980; Krettli, 1982, 1983; Scott & Snary, 1982).

TABLE I

Kinetics of appearance of circulating antibodies measured by CoML and CS and of protection against *T. cruzi* evaluated by the removal of the trypomastigote challenge from circulation (clearance) and survival of the challenged mice

| Days after first *T. cruzi* inoculation* | Reciprocal of antibody titers measured by | | Clearance (%) of the trypomastigote challenge i.v. ($2\text{-}3 \times 10^6$ / An) | Parasitemia on days 6-8 after infection | Total No. mice/ number surviving (%) up to day 8 after challenge |
|---|---|---|---|---|---|
| | CoML | CS | | | |
| 0 | neg. | neg. | None up to 90 min | +++ | 30/0 (0%) |
| 12 | 1:2.5 | neg. | None up to 90 min | 0 | 5/5 (100%) |
| 21 | 1:5 | 1:20 | None up to 90 min | 0 | 5/5 (100%) |
| 31 | 1:320 | 1:40 | Yes (50% $\leqslant$ 90 min) | 0 | 5/5 (100%) |
| 35 | 1:320 | 1:80 | Yes (50% $\leqslant$ 90 min) | 0 | 5/5 (100%) |
| 43 | 1:320 | 1:160 | Yes (90% $\leqslant$ 10 min) | 0 | 5/5 (100%) |
| 48 | 1:320 | 1:320 | Yes (100% $<$10 min) | 0 | 5/5 (100%) |
| 55 | 1:320 | 1:320 | Yes (100% $<$10 min) | 0 | 5/5 (100%) |

CoML = Complement mediated lysis; CS = Conventional serology. Both tests were performed as described in the text.
*Mice were infected with $1\text{-}5 \times 10^3$ trypomastigotes of the Y strain/20 weight i.p., treated once with a subcurative dose of benznidazol and used for the tests.
+++ = about $2\text{-}6 \times 10^6$ trypomastigotes/ml of blood.

We have strong evidences (unpublished) that C factors mediate the *in vivo* clearance. Thus, injection of cobra venon factor in mice chronically infected significantly increases the time needed for parasite clearance although it did not hampere the strong immunity displayed by those animals.

**Mechanism(s) of complement (C) activation by trypomastigotes**

*T. cruzi* epimastigotes but not trypomastigotes have glycoproteins (GP) on their surface which directly activate the C system and result in parasite lysis. Recent data from Sher et al. (1984) indicate that GP 72 (which is a family of structurally related GP expressed on the epimastigotes, absent from blood trypomastigotes and amastigotes, but also expressed on the surface of both insect and cultured derived trypomastigotes) is the major acceptor for $C_3$. However, GP 72 is not required for the regulation of C activation since the metacyclic trypomastigotes treated with pronase loose the GP 72 (Sher et al., 1984) but are converted into activators of the C system (Kipnis et al., 1981).

Trypomastigotes, bound *in vivo* or *in vitro* to specific antibodies, activate the complement system and are extensively lysed as discussed on the previous itens. Both pathways of C activation, the classical and the alternative, participate on this lysis (Krettli, Weisz-Carrington & Nussenzweig, 1979; Budzko, Pizzimenti & Kierszenbaum, 1975). Puzzling enough, the alternative pathway of complement activation has proved to be the most important phenomenon which has now been elucidated as explained.

We have shown (Krettli & Pontes de Carvalho, 1983) that bloodstream trypomastigotes activate $C_3$ since $C_3b$ and inactivated $C_3b$ bound molecules are easily demonstrated on their surface membrane after previous treatment with normal human serum. The $C_3b$ is revealed by anti-$C_3$ serum or monoclonal; by zymosan; and by bovine conglutinine. Furthermore, freshly isolated bloodstream trypomastigotes from immunosuppressed acutely infected mice also carry membrane bound $C_3$ as shown by immunofluorescence techniques (Krettli, 1978; Krettli, Weisz-Carrington & Nussenzweig, 1979). Therefore, the trypomastigotes have a mechanism to interrupt the complement cascade and we have proposed a $C_3$ receptor on the trypomastigote surface membrane (Krettli & Pontes de Carvalho, 1983). A possibility that those parasites, like most mammalian cells, have a decaying accelerating factor (DAF) (Medof, Kinoshita & Nussenzweig, 1984) is another hypothesis to explain the presence of $C_3bi$ on *T. cruzi* trypomastigotes. We have yet no exact conclusion for either hypothesis but curiously enough, treatment of the trypomastigotes with immune $F(ab')_2$ and Fab fragments, from either IgG or IgM (purified from human or mouse sera in the chronic phase of infection) were able to convert them into activators of the C system (Kipnis, Krettli & Dias da Silva, 1985). Since the Fab fragments were capable to prepare trypomastigotes for lysis, participation of the Fc domain in the C activation process has been excluded. It was also shown that the IgG fragments promoted lysis through the activation of the alternative C pathway (ACP). Therefore it is reasonable to accept Kipnis et al. suggestion that the protective antibodies (=lytic antibodies) combine with specific epitopes inhibitors of the ACP, present on the trypomastigote cell surface.

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