Immunology 1985 **56** 227

Antibodies to calmodulin during experimental *Trypanosoma brucei rhodesiense* infections in rabbits

L. RUBEN & C. L. PATTON *Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut, U.S.A.*

Accepted for publication 3 June 1985

Summary. Calmodulin is an intracellular Ca^{2+} receptor protein which regulates a wide variety of enzymatic processes in eukaryotic cells examined in detail. Native calmodulin is not antigenic in rabbits because of its small size, high degree of amino acid sequence conservation and hydrophobicity. African trypanosomes contain a novel calmodulin which is structurally distinct from bovine brain and *Tetrahymena* calmodulins. In the present study, we examine the antibody response towards these calmodulins during chronic *Trypanosoma brucei rhodesiense* infections. Injection of purified typanosome calmodulin into rabbits stimulates the production of specific IgG antibodies which recognize trypanosome, but not bovine brain or *Tetrahymena* calmodulins. By contrast, during chronic *T. brucei* infections in rabbits, antibodies (IgG+IgM+IgA) that recognize trypanosome, *Tetrahymena* and mammalian calmodulins arise. When only IgG antibodies are evaluated from infection sera, the major response is against mammalian and *Tetrahymena* calmodulins. Significantly fewer IgG antibodies are measured in the infection sera which recognize trypanosome calmodulin, while the non-specific control protein, chicken ovalbumin, is not recognized. Peak IgG antibody responses against calmodulin occur between Days 30–34 post-infection. Competition assays indicate that *Tetrahymena* and mammalian calmodulins are recognized at identical epitopes which are distinct from epitopes on trypanosome calmodulin. We conclude that, in the context of chronic *T. brucei* infections in rabbits, antibodies arise which are able to recognize mammalian host calmodulin.

Correspondence: Dr Larry Ruben, Dept. Epidemiology and Public Health, Yale University School of Medicine, 60 College Street, New Haven, CT 06510, U.S.A.

INTRODUCTION

Calmodulin is a multifunctional Ca^{2+}-binding protein which regulates a wide variety of enzymatic processes in response to Ca^{2+} signals (reviewed by Klee & Vanaman, 1982). Calmodulin is a critical regulatory component of all eukaryotic cells examined in detail. Phylogenetic studies demonstrate that calmodulin is highly conserved, exhibiting only five amino acid substitutions in organisms ranging from coelenterates to vertebrates (Klee & Vanaman, 1982). As a consequence of its conserved primary structure, small mass and hydrophobicity, the production of antibodies agianst mammalian calmodulin in rabbits is difficult to accomplish. Only chemically modified vertebrate calmodulins have been successfully demonstrated to be antigenic in rabbits (Van Eldik & Watterson, 1981; Wallace & Cheung, 1979). In contrast with the conserved metazoan calmodulins, we have recently established that three subgenera of African trypanosomes [*Trypanosoma (Trypanozoan) brucei, T. (Nannamonas) congolense* and *T. (Duttonella) vivax*] contain identical calmodulins distinct from all other sources (Ruben, Egwuagu & Patton, 1983; Ruben *et al.*, 1984). Substitutions occur in 17 out of 148 amino acids when compared with bovine brain, and 22 amino

acids when compared with *Tetrahymena* calmodulins (unpublished observations). Therefore, the divergent trypanosome calmodulin is potentially antigenic in the vertebrate host in contrast with the conserved host calmodulin. The following study examines the antibody response of rabbits to isolated unmodified trypanosome calmodulin compared with the response produced when calmodulin is presented together with other trypanosome proteins during chronic infections. We anticipated that antibodies directed against typanosome calmodulin might also recognize epitopes on the host protein. A situation of this nature has been reported during Chagas' disease (Szarfman *et al.*, 1982) and cutaneous leishmaniasis (Avila, Rojas & Rieber, 1984) where a laminin-like protein on the surface of the parasites induces an antibody response which cross-reacts with host basement membranes. The potential of infection sera to interact with host proteins may be of pathological significance.

MATERIALS AND METHODS

Trypanosomes

A triple-cloned Yale Trypanozoan antigenic type 1.1 (YTat 1.1) variant of *Trypanosoma brucei rhodesiense* derived from UGANDA/60/TREU 164 (ETat 3) was used throughout this study (Ruben *et al.*, 1983). Stabilates were maintained in the vapour phase of a liquid N_2 freezer.

Calmodulin

Calmodulin was purified from homogenates of bovine brain, trypanosome and *Tetrahymena* using a combination of DE-52 and phenyl-Sepharose hydrophobic affinity chromatography (Ruben *et al.*, 1983). *Tetrahymena* calmodulin was isolated with the addition of a trichloroacetic acid precipitation step (Jamieson & Frazier, 1983) prior to DE-52 chromatography. Bovine brain was used instead of rabbit calmodulin due to the large quantities obtainable from this source. The primary sequence of rabbit skeletal and bovine calmodulins are identical, with the possible exception of three amidation states which cannot be unambiguously assigned (Nairn, Grand & Perry, 1984). There is no evidence to suggest that these potential differences affect antigenic properties of the proteins. In general, mammalian calmodulins are presumed to be structurally and functionally identical (Klee & Vanaman, 1982).

Immunization

Purified trypanosome calmodulin was emulsified in 100-μg aliquots with Freund's complete adjuvant, and 50 μg were injected into each popliteal lymph node of 2–3 kg New Zealand rabbits. Animals were boosted subcutaneously four times at 3-week intervals with 100 μg calmodulin in Freund's incomplete adjuvant and exsanguinated 2 weeks after the last boost. The 50% ammonium sulphate fraction from serum was obtained. Alternatively, 10 μg of purified SDS-denatured trypanosome calmodulin were separated on 15% SDS-PAGE using the buffers of Laemmli (1970). Acrylamide slices containing calmodulin were sheared by repeated syringe passage and injected i.p. into each of four BALB/c mice. Each mouse was boosted once by i.p. injection with 20 μg native calmodulin in Freund's incomplete adjuvant. Mice were bled 2 weeks after the boost and sera were collected.

Infection

Three separate 2–3 kg New Zealand white rabbits (R_{inf} 1, R_{inf} 2 and R_{inf} 3) were each infected i.v. with 5×10^7 cells of YTat 1.1. Serum samples were obtained at intervals throughout the infection.

Radioimmunoassays

Antigen to be tested was dissolved in 0·05 M carbonate buffer, pH 9·6, and added in 1-μg aliquots to polyvinyl chloride microtitre plates (Dynatech Laboratories, Alexandria, VA). Approximately 27 ng of calmodulin were bound under these conditions as determined with [^{125}I]calmodulin of known specific activity (Amersham, Arlington Heights, IL). Following a 2-hr incubation at 37°, the plates were washed with radioimmunoassay buffer (RIA buffer) consisting of 50 mM Na phosphate, 100 mM NaCl, 1% bovine serum albumin and 0·02% Na azide, pH 7·5. Antibody dilutions were added in RIA buffer and incubated at 37° for 2 hr. When total immunoglobulin was monitored, the plates were washed three times in RIA buffer and then incubated with secondary goat anti-rabbit immunoglobulins (IgG + IgM + IgA; Cappel Laboratories, Malvern, PA). Following three more washes, [^{125}I]protein A (0·15 μCi; 20 Ci/mg) was added and further incubated at 37° for 2 hr. Unbound [^{125}I]protein A was removed by washing four times in RIA buffer. Individual microtitre wells were excised and radioactivity was detected with a γ-counter (Packard, Downers Grove, IL). IgG antibodies were detected with [^{125}I]protein A by omitting the secondary antibody.

Competition assays were run by adding 1 μg bovine brain calmodulin to each well of a polyvinyl chloride microtitre plate as described above. Serum from R_{inf} 1, Day 38 post-infection, or R_{inf} 2, Day 43 post-infection, was diluted 1:20 with RIA buffer and incubated overnight at 4° with increasing amounts of calmodulins from different sources or with chicken ovalbumin. The preabsorbed antisera were added directly to the microtitre plates, incubated and washed as described above. Bound antibody was detected with [^{125}I]protein A. Maximum counts in the absence of competing antigen were 4500 c.p.m. Background was 150 c.p.m.

Western analysis

Calmodulins from different sources were separated on 15% SDS-containing gels with the buffers of Laemmli (1970), and electrophoretically transferred to nitrocellulose sheets as described by Burnette (1981). The nitrocellulose was washed with 20 mM Tris-HCl, 50 mM NaCl, pH 7·5 (TBS), blocked in the same buffer containing 3% gelatin and incubated with rabbit antiserum (1:20 dilution) overnight at 25° with shaking. Following three washes in TBS containing 0·05% Tween-20, bound antibody was visualized with horseradish peroxidase-conjugated goat anti-rabbit IgG (BioRad, Richmond, CA) and 4-chloro-naphthol as colour reagent.

RESULTS

Antibody response of rabbits and mice to purified trypanosome calmodulin

Antigenic properties of trypanosome calmodulin were evaluated by injecting purified protein in a native or denatured state into rabbits or mice. As can be seen in Fig. 1, in contrast to the situation with the mammalian protein (Van Eldik & Watterson, 1981; Wallace & Cheung, 1979), trypanosome calmodulin is antigenic. Injection of native trypanosome calmodulin into each of two rabbits resulted in antibodies in hyperimmune sera which were specific for trypanosome calmodulin (Fig. 1A). Antibody titres were approximately 10^5. No cross-reactivity with bovine brain or *Tetrahymena* calmodulins was measured. The inset to Fig. 1A shows the pattern of cross-reactivity by Western procedures. The converse situation was also true: rabbit antisera against *Tetrahymena* calmodulin did not cross-react with trypanosome or brain calmodulins (Yasuhiro *et al.*, 1982).

When mice were immunized with SDS-denatured trypanosome calmodulin, IgM and IgG antibodies were produced (Fig. 1B). Titres of approximately 10^4 and 10^5, respectively, were measured against native trypanosome calmodulin. By contrast with the situation in rabbits, mouse IgG antibodies recognized both trypanosome and brain calmodulin (Fig. 1C), indicating that conditions of antigen presentation and recipient species exist which induce cross-reacting antibodies.

Calmodulin antibodies in sera from chronically infected rabbits

In order to evaluate antigenic properties of released trypanosome calmodulin during the course of chronic *T. brucei rhodesiense* infection, 5×10^7 organisms of YTat 1.1, a pleomorphic clone, were injected i.v. into each of three rabbits (R_{inf} 1, R_{inf} 2, and R_{inf} 3). Blood samples were obtained periodically and sera were analyzed by solid-phase RIA for antibodies against trypanosome, bovine brain or *Tetrahymena* calmodulins and an unrelated control protein, chicken ovalbumin. Immunoglobulins that recognized these proteins were measured using [^{125}I]protein A and goat anti-rabbit immunoglobulin (IgG + IgM + IgA). As can be seen in Fig. 2A–C, antibodies were detected which recognized calmodulins from each source tested. Peak antibody responses occurred between Days 12 and 16 and plateaued thereafter. In the case of R_{inf} 1 (Fig. 2A), antibodies at Day 16 post-infection against trypanosome calmodulin, bovine calmodulin, *Tetrahymena* calmodulin or ovalbumin were, respectively, 18, 19, 12 and 8-fold greater than the corresponding preinfection serum response. With R_{inf} 2 (Fig. 2B), at Day 16 post-infection, antibodies were, respectively, 10, 14, 7 and 1.4-fold greater than the corresponding preinfection serum response. R_{inf} 3 (Fig. 2C) produced a response at Day 16 that was, respectively, 25, 10, 20 and 3.3-fold greater than was observed with the corresponding preinfection serum. Therefore, infected rabbits generated antibodies which recognized trypanosome, bovine brain and *Tetrahymena* calmodulins. An increase in ovalbumin antibodies was observed, despite the fact that no chicken antigens were injected. The response to calmodulin in all cases exceeded the non-specific response to ovalbumin.

The IgG component of the total antibody response was determined using the assay employed in Fig. 2A–C, except that the secondary goat IgG anti-rabbit immunoglobulin was omitted. In contrast with Fig. 2A–C, where all serum immunoglobulin classes were

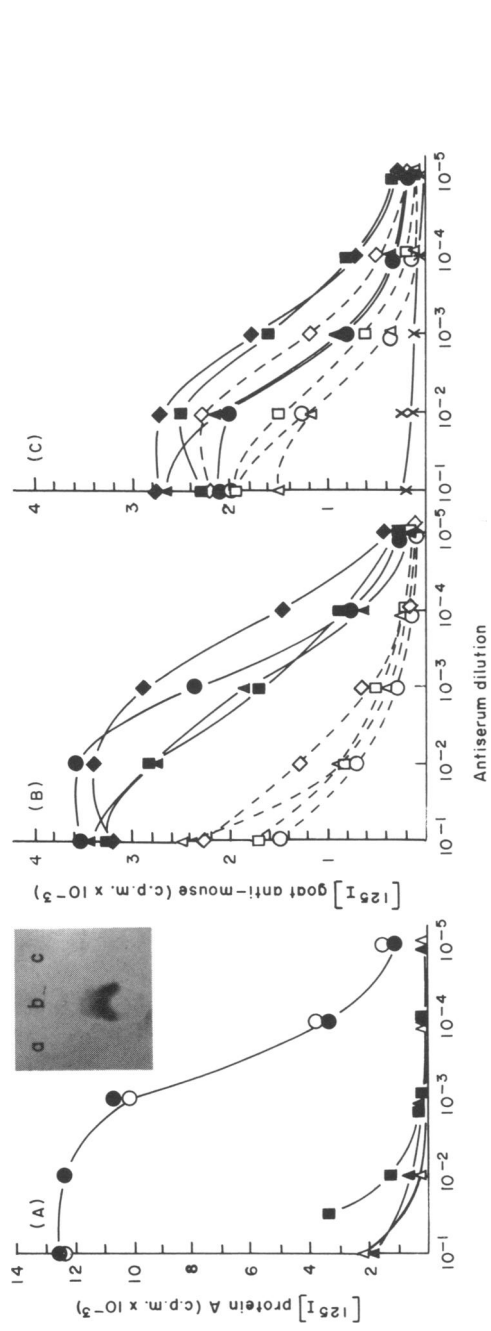

Figure 1. Polyclonal antibodies raised in rabbits and mice against tryponosome calmodulin. (A) The ability of rabbit immune serum to recognize trypanosome calmodulin (●, ○), bovine brain calmodulin (▲) or *Tetrayhena* calmodulin (■) was tested by solid-phase radioimmunoassay. Preimmune serum binding to trypanosome calmodulin was also tested (△). The inset shows a Western analysis of rabbit antibody binding to bovine brain calmodulin (Lane a), trypanosome calmodulin (Lane b) or *Tetrahymena* calmodulin (Lane c). The *Tetrahymena* calmodulin used in these studies was kindly provided by Dr T. Vanaman, University of Kentucky. (B) Polyclonal antibodies against trypanosome calmodulin were raised in each of four mice. The ability of different antibody classes to recognize trypanosome calmodulin was determined by the procedures outlined in Fig. 1A, except that bound antibodies were visualized with [^{125}I]rabbit anti-mouse IgG (●, ♦, ■, ▲; solid lines), or, correspondingly, with rabbit anti-mouse IgM (○, ◊, □, △; dotted lines). (C) IgG antibodies from each mouse were examined for their specificity towards trypanosome calmodulin (●, ♦, ■, ▲; solid lines) or bovine brain calmodulin (○, ◊, □, △; dotted lines). Preimmune serum against trypanosome calmodulin (×; solid line) was also determined.

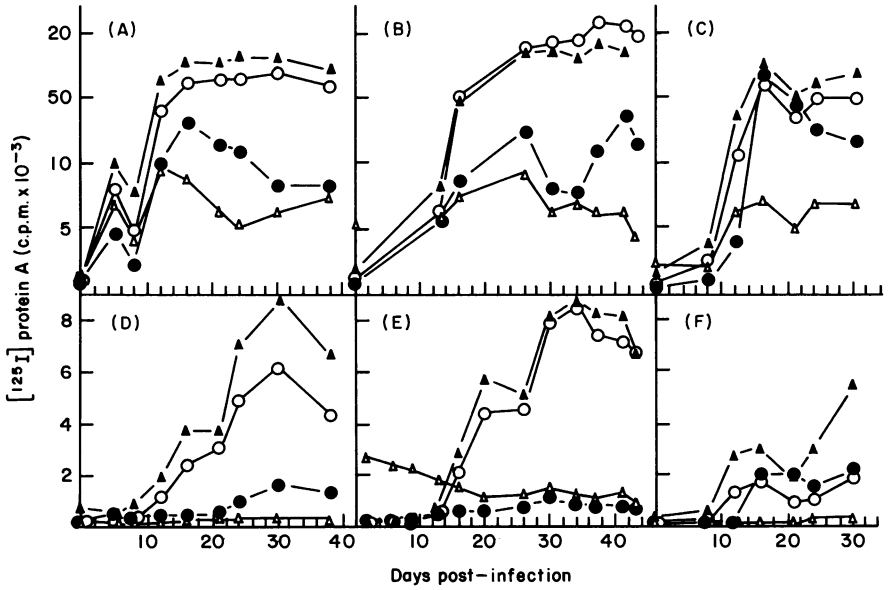

Figure 2. Production of calmodulin antibodies by rabbits chronically infected with *T. brucei rhodesiense*. Three separate 2–3 kg rabbits ($R_{inf}1$, $R_{inf}2$, and $R_{inf}3$) were each infected with 5×10^7 cells of YTat1.1 by i.v. infection. Blood samples were taken on various days post-infection as indicated. Antibody response of $R_{inf}1$ (A,D), $R_{inf}2$ (B,E) and $R_{inf}3$ (C,F) towards trypanosome calmodulin (●), bovine brain calmodulin (○). *Tetrahymena* calmodulin (▲) or chicken ovalbumin (△) was determined by solid-phase radioimmunoassay. Each serum sample was tested at a dilution of 1 : 15. The serum immunoglobulin responses were determined (Panels A, B and C) by the addition of secondary goat anti-rabbit immunoglobulins following incubation with test serum. The IgG responses (D,E,F) were measured without secondary antiserum. $R_{inf}1$ and 2 were exsanguinated on Days 38 and 43, respectively. $R_{inf}3$ died on Day 36 post-infection.

measured, no increase in anti-ovalbumin was detected. However, IgG antibodies against calmodulin were found. These antibodies peaked between Days 30 and 34 post-infection (Fig. 2D–F). Interestingly, sera from $R_{inf}1$ and 2 developed greater IgG responses against bovine brain and *Tetrahymena* calmodulins than against trypanosome calmodulin. $R_{inf}3$ sera tested equally against all of the calmodulins. The responses of sera from each rabbit to bovine brain or *Tetrahymena* calmodulins were significantly greater than those observed against ovalbumin.

Relationship of calmodulin epitopes recognized by infection sera

Since the amount of IgG antibodies that recognized brain and *Tetrahymena* calmodulins greatly exceeded the amount that recognized trypanosome calmodulin in $R_{inf}1$ and 2, it was important to determine whether antibodies in sera from these rabbits bound to cross-reacting determinants among these proteins. Consequently, a solid-phase RIA competition assay was developed. As shown in Fig. 3A–B, ovalbumin did not prevent binding of antisera to bovine brain calmodulin immobilized on microtitre plates. By contrast, exogenous calmodulins from brain, *Tetrahymena* and, to a lesser extent, from trypanosomes, did prevent antibody binding. In the case of $R_{inf}1$ (Fig. 3A), where maximal inhibition occurred when 83% of the antibody was displaced, half maximal inhibition required 0·38, 0·58 and 2·6 μg of brain, *Tetrahymena* and trypanosome calmodulins, respectively. For $R_{inf}2$ (Fig. 3B), where maximal inhibition occurred with 70% of the antibody displaced by brain and *Tetrahymena* calmodulins and 54% displaced by trypanosome calmodulin, half maximal inhibition required 0·9, 0·9 and 4·0 μg calmodulins, respectively. Therefore, IgG antibodies which recognize brain calmodulin cross-reacted equivalently with *Tetrahymena* calmodulin. However, it took 4·4–6·8 times as much competing trypanosome calmodulin to half maximally inhibit the interaction (Fig. 3A–B). We conclude that epitopes on brain calmodulin differ from those recognized on trypanosome calmodulin in these rabbits.

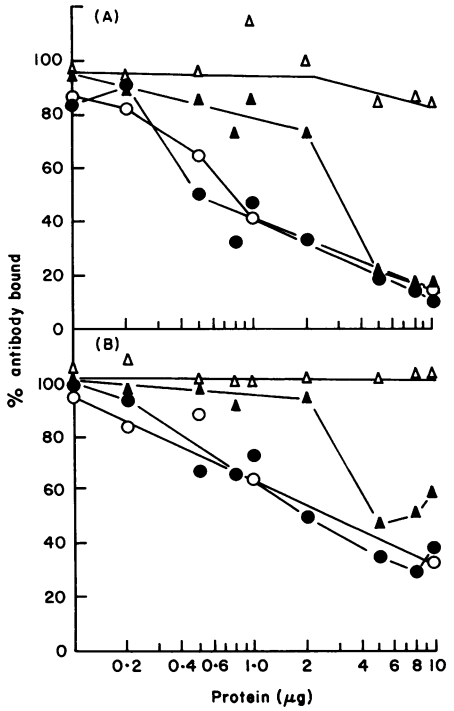

Figure 3. Competitive antibody binding assay. Bovine brain calmodulin (1 μg per well) was bound to polyvinyl chloride microtitre plates. Serum samples from (A) $R_{inf}1$, Day 38 post-infection or (B) $R_{inf}2$, Day 43 post-infection were diluted 1:20 with RIA buffer and incubated overnight at 4° with increasing amounts of trypanosome calmodulin (▲), bovine brain calmodulin (●), *Tetrahymena* calmodulin (○) or chicken ovalbumin (△). Indicated amounts of competing proteins were added per 0·1 ml reaction mixture. The preabsorbed antiserum was added directly to the microtitre plate and detected with [^{125}I]protein A. Maximum counts in the absence of competing antigen were 4500 c.p.m. Background was 150 c.p.m.

DISCUSSION

During African trypanosomiasis, the predominant antibody response is directed against a 15-nm thick variant surface glycoprotein (VSG) which envelops the parasite (Borst & Cross, 1982). Undulating parasitaemias develop when VSG-directed antibodies destroy major antigenic types followed by proliferation by trypanosomes of different antigenic types (Vickerman & Barry, 1982). A consequence of the humoral responses during trypanosomiasis is immune complex-mediated damage to nerve, heart, muscle, kidney and lung tissues (reviewed in Mansfield, 1981). Autoimmune antibodies specific for ganglion cells (Poltera *et al.*, 1980), liver (Seed & Gam, 1967), erythrocytes (Kobayashi, Tizard & Woo, 1976), DNA (Lindsley, Kysela & Sternberg, 1974), fibrin/fibrinogen (Boreham & Facer, 1974) and intermediate filaments (Mortazavi-Milani, Facer & Holborow, 1984) have been described. Although humoral responses to trypanosome antigens are envisaged to play a role in pathogenesis, few trypanosome antigens have been specifically identified and, with the exception of VSG, none has been characterized. In the present study, we examine the development of antibodies against calmodulin, a well-characterized protein of the host and the parasite during chronic *T. brucei* infections.

Calmodulin is a highly conserved regulatory protein which represents a significant fraction of total cellular protein [0·3% of trypanosome protein (Ruben *et al.*, 1983) and 0·1–1% of host protein (Klee & Vanaman, 1982)]. In spite of the observation that mammalian calmodulin is poorly antigenic in rabbits, the total immunoglobulin response (IgG + IgM + IgA) during trypanosome infections includes antibodies that recognize trypanosome, as well as bovine brain and *Tetrahymena* calmodulins (Fig. 2A–C). These antibodies appear to be of the IgM class as derived by (i) their early onset and (ii) their different pattern of appearance from IgG antibodies against the same proteins as determined by radioimmunoassay (Fig. 2D–F). Some of the total immunoglobulin response may result from non-specific activation of B-cell clones (Greenwood, 1974). The rise in serum antibodies against chicken ovalbumin is best explained in this way (Fig. 2A–C). However, the production of IgG antibodies against mammalian calmodulin may represent a specific response to the host antigen. To begin with, these antibodies appear late in infection and follow the peak in IgM antibodies as is expected of a specific antibody response (Fig. 2D–F). The antibodies produced recognize specific regions of the host calmodulin which are identical with determinants on *Tetrahymena* calmodulin but different from determinants on trypanosome calmodulin (Fig. 3A–B). Finally, different rabbits produce infection sera which are similar in their ability to recognize cross-reacting determinants on host and *Tetrahymena* calmodulins. Although it is unclear how they are induced, IgM and IgG antibodies to host and typanosome calmodulin do develop during infection. Infection sera and sera from rabbits or mice injected with the pure protein are distinct from one another based upon the specificity of their response towards calmodulins from different

sources (Fig. 1A–C). The production of antibodies against calmodulin during trypanosomiasis may resemble the production of antibodies against laminin during Chagas' disease and leishmaniasis (Szarfman *et al.*, 1982; Avila *et al.*, 1984). Laminin antibodies may result from an immune response directed against the parasite laminin-like protein or the host basement membranes. We are testing the suggestion that host calmodulin in association with foreign trypanosome-binding proteins may become antigenic. We have previously shown that brain calmodulin binds trypanosome proteins (Ruben *et al.*, 1984). Alternatively, deregulation of the immune system as a consequence of the disease may result in the producton of antibodies against the conserved mammalian calmodulin. These data demonstrate that, during the course of chronic typanosome infections in rabbits, antibodies are produced which recognize mammalian host calmodulin on epitopes distinct from those present on trypanosome calmodulin.

ACKNOWLEDGMENTS

A portion of this work was carried out at the International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya. The authors wish to thank Alfred Adema and Naseem Saigar at ILRAD, and Diane Grabowski at Yale University, for technical assistance. Eta Kaplan is thanked for typing the manuscript.

This work was supported by USPHS, NIH grant nos T32 AI-07136 and RO1 AI-15742.

REFERENCES

AVILA J.L., ROJAS M. & RIEBER M. (1984) Antibodies to laminin in American cutaneous leishmaniasis. *Infect. Immun.* **43,** 402.

BOREHAM P.F.L. & FACER C.A. (1974) Autoimmunity in trypanosome infections. II. Anti-fibrin/fibrinogen (Anti-F) autoantibody in *Trypanosome (Trypanozoan) brucei* infections of the rabbit. *Int. J. Parasitol.* **4,** 601.

BORST P. & CROSS G.A.M. (1982) Molecular basis for trypanosome antigenic variation. *Cell*, **29,** 291.

BURNETTE W.N. (1981) 'Western blotting': electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulase and radiographic detection with antibody and radioiodinated protein A. *Analyt. Biochem.* **112,** 195.

GREENWOOD B.M. (1974) Possible role of B-cell mitogen in hypergamma-globulinaemia in malaria and typanosomiasis. *Lancet*, **i,** 435.

JAMIESON G.A. & FRAZIER W.A. (1983) Dictyostelium calmodulin: affinity isolation and characterization. *Arch. Biochem. Biophys.* **227,** 609.

KLEE C.B. & VANAMAN T.C. (1982) Calmodulin. *Adv. Prot. Chem.* **35,** 213.

KOBAYASHI A., TIZARD I.R. & WOO P.T.K. (1976) Studies on the anemia in experimental African trypanosomiasis. The pathogenesis of the anemia in calves infected with *Trypanosoma congolense*. *Am. J. trop. Med. Hyg.* **25,** 401.

LAEMMLI U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*, **227,** 680.

LINDSLEY H.B., KYSELA S. & STERNBERG A.D. (1974) Nucleic acid antibodies in African trypanosomiasis: studies in rhesus monkeys and man. *J. Immunol.* **113,** 1921.

MANSFIELD J.M. (1981) Immunology and immunpathology of African trypanosomiasis. In: *Parasitic Diseases, The Immunology* (ed. J. M. Mansfield), p. 167. Marcel Decker Inc., New York.

MORTAZAVI-MILANI S.M., FACER C.A. & HOLBOROW E.J. (1984) Induction of anti-intermediate filament antibody in rabbits experimentally infected with *Trypanosoma brucei brucei*. *Immunology*, **52,** 423.

NAIRN A.C., GRAND R.A.J. & PERRY S.V. (1984) The amino acid sequence of rabbit skeletal muscle calmodulin. *FEBS Lett.* **167,** 215.

POLTERA A.A., HOCHMANN A., RUDIN W. & LAMBERT P.H. (1980) *Trypanosoma brucei brucei*: a model for cerebral trypanosomiasis in mice—an immunological, histological and electron microscopic study. *Clin. exp. Immunol.* **40,** 496.

RUBEN L., EGWUAGU C. & PATTON C.L. (1983) African trypanosomes contain calmodulin which is distinct from host calmodulin. *Biochim. biophys. Acta*, **758,** 104.

RUBEN L., STRICKLER J.E., EGWUAGU C. & PATTON C.L. (1984) Structural and biologic properties of calmodulin from African trypanosomes. In: *Molecular Biology of Host–Parasite Interactions*, (eds N. Agabian and H. Eisen), p. 267. Alan R. Liss Inc., New York.

SEED J.R. & GAM A.A. (1967) The presence of antibody to a normal rabbit liver antigen in rabbits infected with *Trypanosoma gambiense*. *J. Parasitol.* **53,** 946.

SZARFMAN A., TERRANOVA V.P., RENNARD S.I., FOIDARD J.M., LIMA M.F., SCHEINMAN J.I. & MARTIN G.R. (1982) Antibodies to laminin in Chagas' disease. *J. exp. Med.* **155,** 1161.

VAN ELDIK L.J. & WATTERSON D.M. (1981) Reproducible production of antiserum against vertebrate calmodulin and determination of the immunoreactive site. *J. biol. Chem.* **256,** 4205.

VICKERMAN K. & BARRY J.D. (1982) African trypanosomiasis. In: *Immunology of Parasitic Infections*, (eds S. Cohen and K.S. Warren), p. 204. Blackwell Scientific Publications, Oxford.

WALLACE R.W. & CHEUNG W.Y. (1979) Calmodulin production of an antibody in rabbit and development of radioimmunoassay. *J. biol. Chem.* **254,** 6564.

YASUHIRO S., OHNISHI K., HIRABAYASKI T. & WATANABE Y. (1982) *Tetrahymena* calmodulin: characterization of an anti-*Tetrahymena* calmodulin and the immunofluorescent localization in Tetrahymena. *Exp. Cell Res.* **137,** 1.