Fl-160

A Surface Antigen of *Trypanosoma cruzi* that Mimics Mammalian Nervous Tissue

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It has long been speculated that many autoimmune diseases are the result of infectious agents. Examples of autoimmune diseases believed to have an infectious "trigger" include rheumatic heart disease (group A streptococcus) (1), celiac disease (adenovirus), ankylosing spondylitis, and Reiter's syndrome (klebsiella, yersinia, and shigella) (2). For example, in rheumatic heart disease, group A streptococcus, which causes this disease, has been shown to have crossreactive antigens with valvular tissue. Thus, by molecular mimicry, this pathogen sets up the autoimmune response that leads to the destruction of heart valves (1). Despite the importance of understanding autoimmune diseases, there are few experimental models of autoimmune disease induced by an infectious agent.

Chagas' disease, caused by the intracellular parasite *Trypanosoma cruzi*, is an excellent model for autoimmune disease induced by an infectious agent. The chronic disease is characterized by rich inflammatory infiltrates in myocardial and nervous tissues, with few, if any, demonstrable parasites. Autoantibodies can be found in sera from Chagas' patients (3, 4). Selected mAbs to *T. cruzi* have been found to crossreact with nervous tissue antigens (5–8). T cells directed against cardiac and nervous tissue antigens have been found in mice with experimental Chagas' disease (9–13). T cells directed against *T. cruzi* antigens which crossreact with nervous tissue, have been found to recreate nervous tissue pathology when transferred to naive animals (12, 13). This has led to the hypothesis that *T. cruzi* antigenically mimics host tissues that are damaged by inflammatory cells in chronic disease. It is important to characterize these crossreactive antigens in order to understand the breakdown of immunoregulation that allows for autoimmunity. Yet, the molecular nature of crossreactive antigens in *T. cruzi* and nervous tissues is still poorly understood.

We used DNA cloning techniques to characterize a *T. cruzi* surface antigen that crossreacts with mammalian tissues. We have cloned DNA from *T. cruzi* that expresses a protein corresponding to a 160-kD protein found on the surface of the trypanosome, overlying the flagellum. Antibodies to this protein crossreact with a 48-kD mammalian nervous tissue protein. Thus, the antigen is a candidate antigen for autoimmune mimicry leading to nervous tissue pathology.

Materials and Methods

T. cruzi.   *T. cruzi* CL strain, used throughout, was "subclone three" described in reference 14, and was maintained in liver infusion/tryptone (LIT) medium with 10% fetal bovine medium as the epimastigote form. Trypomastigotes were obtained from culture supernatants of rat 3T3 cells infected with trypomastigotes and commonly contained 10-40% amastigote forms. 8-wk-old female C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME) were infected with *T. cruzi* by intraperitoneal injection of 5×10^4 tissue culture-grown trypomastigotes. Infection was confirmed by finding trypomastigotes in blood films at 5-8 d after infection, and mice were considered chronically infected at 3 mo after infection. Chronically infected mouse sera were absorbed with 20% (vol/vol) formalin-fixed *Escherichia coli*.

DNA Cloning and Analysis.   DNA was selected by eluting 4-8 kb sonicator sheared *T. cruzi* epimastigote DNA from an agarose gel, repairing, and cloning into λ GT11 using standard techniques (15, 16). The gFl-160 DNA clone described here was one of many clones expressing fusion proteins reactive with chronically infected mouse sera. The gFl-160 insert DNA was subcloned into Bluescribe plasmid (Stratagene Inc., La Jolla, CA). Probes for Northern hybridization were agarose gel–purified restriction fragments of CsCl-purified plasmid DNA, labeled by random priming in the presence of α-[^{32}P]CTP (16, 17). RNA for Northern hybridization was purified by cesium chloride gradient centrifugation of guanidinium-lysed trypomastigotes and epimastigotes. 5 μg per lane was subjected to electrophoresis in formaldehyde agarose gels and transferred onto nylon membranes (Genescreen Plus; New England Nulear, Boston, MA) (16). Northern hybridization was carried out at 42°C using standard techniques (16), and washed at high stringency (65°C, 0.1 × SSPE).

The Fl-160 DNA was subcloned into a plasmid (Glutagene; a generous gift of G. F. Michell, D. B. Smith, and the AMRAD Corp., Ltd., Abbotsville, Australia) expressing the FL-160 protein as a fusion to *Schistosoma japonicum* glutathione-S-transferase (GST)[1] (18). This recombinant fusion protein was purified from isopropylthiogalactoside (Sigma Chemical Co., St. Louis, MO) induced bacterial lysates using affinity columns of glutathione-agarose (Sigma Chemical Co.) (18).

Antibody Techniques.   100 μg of affinity-purified recombinant antigen emulsified in Freunds' adjuvant (first complete, then incomplete) was injected monthly intramuscularly in a NZW rabbit. After three boosts, the resultant antiserum was affinity purified by passing 15 cc of sera over a 5-ml column of 5 mg of recombinant GST-cyanogen bromide linked to Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) to deplete the antiserum of anti-GST antibodies. The column was washed extensively and the anti-GST antibodies (used as a control in many experiments) were eluted with 3 M NH₄SCN in PBS. Then, anti-Fl-160 antibodies were prepared similarly from the anti-GST-depleted serum using a column of 3 mg of Fl-160-GST fusion protein linked to CNBr-Sepharose 4B. *T. cruzi* and tissue extracts were prepared as previously reported (13). ELISAs were performed by coating plates with 10 μg/ml of antigens in 50 mM tris, pH 9.3, overnight at 4°C, quenching with 1 mg/ml BSA, exposing to antibodies, components of an anti–rabbit avidin-biotin-peroxidase kit (Vector Laboratories., Inc., Burlingame, CA), developed with 2.2'-Azino-bis(3-ethyl)-azoline-6-sulfonic acid (Sigma Chemical Co.), and read on an automated ELISA reader (Bio-Tek Instruments, Inc., Burlington, VT). Western blots were performed using PAGE according to Laemmli (19) and electroblotted onto nitrocellulose (Schleicher & Schuell, Inc., Keene, NH) (20), treated with 5% nonfat dry milk, exposed to antibodies, and developed with avidin-biotin-peroxidase and diaminobenzidine (Sigma Chemical Co.) or with ^{125}I protein A (New England Nuclear). Immune staining was performed on tissues and parasites fixed in 4% paraformaldehyde/PBS at 4°C, exposed to 1 mg/ml BSA, antibodies, and then goat F(ab')² FITC-labeled anti-rabbit IgG (Tago Inc., Burlingame, CA) or anti-rabbit avidin-biotin-peroxidase developed with diaminobenzidine. Parasites were surface labeled and tissue extracts were labeled with ^{125}I using Iodogen (Pierce Chemical Co., Rockford, IL) bound to glass tubes (21). Parasites were lysed with 0.5% NP-40/PBS/0.5 mM PMSF/0.5% aprotinin (Sigma Chemical Co.). Immu-

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[1] *Abbreviations used in this paper:* β-gal, *E. coli* β-galactosidase encoded by λ GT11; CIMS, chronically infected mouse sera; GST, *S. japonicum* glutathione-S-transferase.

noprecipitation was accomplished by incubation with 1 µg/ml antibody, followed by protein A-Sepharose 4B (Pharmacia Fine Chemicals).

Results

We began our studies by making an expression library of randomly sheared *T. cruzi* DNA in λ GT11 and screening with sera from chronically infected mice. This sera has been previously shown to react with mammalian tissues, as well as *T. cruzi* antigens. From nine clones orginally chosen, we have focussed our studies on one, gFl-160, which codes for a crossreactive protein. We have not yet characterized the other eight to know, if they too, code for crossreactive proteins. We will show that the gFl-160 DNA insert produces a recombinant protein (Fl-160) that corresponds to a 160-kD protein found on the surface of *T. cruzi* trypanosomes, overlying the flagella. Antibodies directed to Fl-160 crossreact with nervous tissues found at sites of chronic pathology, recognizing a 48-kD protein.

gFl-160 Insert Encodes a Polypeptide Recognized by Chronically Infected Sera. The gFl-160 insert in the λ GT11 vector produces a fusion protein of 180 kD that reacts with chronically infected mouse sera (CIMS) (Fig. 1). Of this, 118 kD is β-gal and the remaining 62 kD of the fusion protein is encoded by the Fl-160 DNA. β-gal or other products of the *E. coli* lysate did not react with CIMS. Uninfected mouse sera does not react with Fl-160 fusion protein (not shown).

To purify the Fl-160 protein, the insert was subcloned into Glutagene, a plasmid vector that expresses DNA fused to *S. japonicum* GST (18). This allowed purification of the Fl-160-GST fusion protein from bacterial lysates in a single step by affinity chromatography on glutathione-agarose. By Coomassie staining (Fig. 1), there are two major polypeptides of Fl-160-GST with molecular masses of 88 and and 83 kD, whereas the recombinant GST protein is 27 kD. This agrees with the λ GT11-derived estimate; that is the Fl-160 DNA codes for an ∼60-kD polypeptide. Fig. 1 shows CIMS reacts with the Fl-160-GST fusion protein. There are a number of smaller molecular weight bands in the purified Fl-160-lysate that are immunoreactive as well. These likely represent degradation products. Affinity-purified anti-Fl-160 antibodies
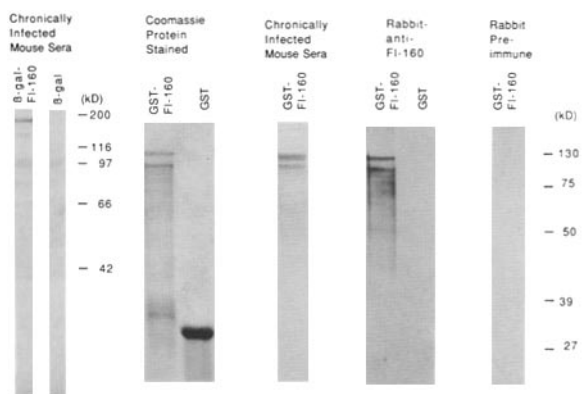

FIGURE 1. Western blot of recombinant proteins. The two left lanes are 50 µg/lane isopropylthiogalactoside-induced *E. coli* RY1089 lysate of λ GT11 with gFl-160 insert (Fl-160-β-gal) and no insert (β-gal) reacted with chronically infected mouse serum (1:200 dilution). The six right lanes are 2 µg/lane GST recombinant proteins, affinity purified on glutathione-agarose, either expressed with gFl-160 insert (Fl-160-GST) or no insert (GST). These lanes are stained with coomassie brilliant blue or reacted with chronically infected mouse serum or affinity-purified rabbit anti-Fl-160 antibodies (1 µg/ml) or rabbit preimmune serum (1:100) preabsorbed with *E. coli*, and all are developed with the avidin-biotin-peroxidase-diaminobenzidine system.

react with Fl-160-GST, but not GST alone (Fig. 1). Affinity-purified anti-GST antibodies react with both Fl-160-GST and GST (not shown). Preimmune rabbit sera does not react with either recombinant protein (Fig. 1).

Anti-Fl-160 Antibodies Immunoprecipitate a 160-kD Surface Protein that Is Associated with the Flagellar Apparatus of Trypomastigotes.   We used indirect immunofluorescence to demonstrate the antigen on parasites. Affinity-purified anti-Fl-160 antibodies stain *T. cruzi* trypomastigotes linearly along the entire length of the flagellum (Fig. 2). The anti-Fl-160 antibodies did not stain epimastigotes (the insect form) or amastigotes (the intracellular form). Control anti-GST antibodies gave no staining with any of these forms.

The anti-Fl-160 also gave strong immunofluorescence with infected 3T3 cells (Fig. 2), but not with uninfected cultures. Most of the fluorescence appeared to come from the surface of the cells, and appeared in a beaded or granular fashion. Interestingly,

Immunofluoresence

Anti-Fl-160
Trypomastigotes

Anti-GST
Trypomastigotes

Anti-Fl160
Epimastigotes

Anti-Fl-160
T. cruzi   Infected 3T3 cells
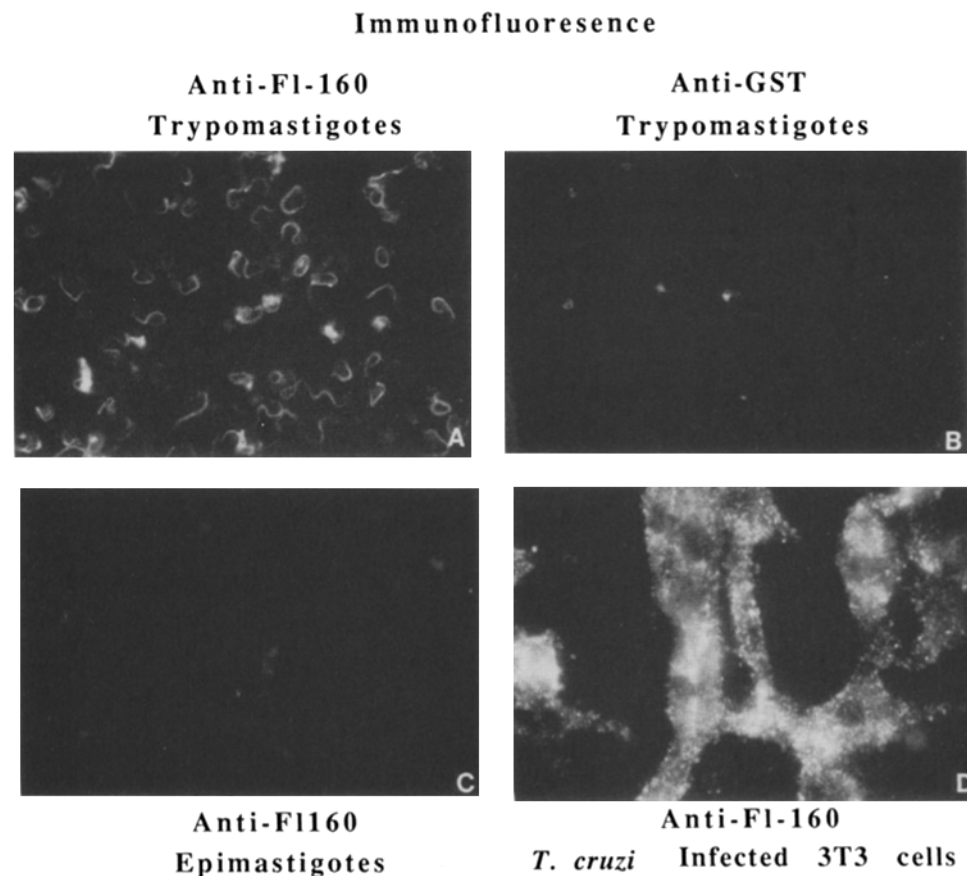
FIGURE 2.   Indirect immunofluorescence shows anti-Fl-160 antibodies react with trypomastigote flagellar membranes and *T. cruzi*–infected 3T3 cells. Parasites and cells are stained with 1 μg/ml anti-Fl-160 or anti-GST and FITC-F(ab')2 anti–rabbit IgG. (*A*) *T. cruzi* trypomastigotes with anti-Fl-160. (*B*) *T. cruzi* trypomastigotes with anti-GST. (*C*) *T. cruzi* epimastigotes with anti-Fl-160. (*D*) 3T3 cells infected with *T. cruzi* trypomastigotes after 5 d in culture.

all cells in the infected culture stained, even cells that appeared to have no parasites intracellularly. No immunofluorescence was seen with control anti-GST antibodies. It seems likely, therefore, that the Fl-160 antigen is absorbed onto uninfected cells.

Anti-Fl-160 antibodies bind or immunoprecipitate a 160-kD surface protein on trypomastigotes. Fig. 3 shows a Western blot developed with anti-Fl-160 antibodies, showing a broad band at 160 kD in the trypomastigote lysate. This 160-kD band was not found with control anti-GST antibodies (Fig. 3), or by anti-Fl-160 antibodies on uninfected 3T3 cell lysates (not shown). In other experiments (not shown), the anti-Fl-160 antibodies did not bind epimastigote lysates. Anti-Fl-160 antibodies, selected from chronically infected mouse sera with Fl-160-β gal fusion protein bound to nitrocellulose, also reacted with the 160-kD protein in trypomastigote lysate Western blots (not shown). A 160-kD protein was immunoprecipitated from lysates of ^{125}I surface-labeled trypomastigotes with anti-Fl-160 antibodies (Fig. 3), demonstrating the protein to be on the surface. Control anti-GST antibodies gave no detectable precipitation.

Anti-Fl-160 Antibodies Crossreact with Mammalian Nervous Tissues. We first detected crossreactivity of anti-Fl-160 antibodies with mammalian antigens in the ELISA. Fig. 4 shows that affinity-pure anti-Fl-160 antibodies react strongly with Fl-160-GST fusion protein, but not with GST, as expected. Anti-GST antibodies react with both of these recombinant proteins because both contain GST epitopes. Anti-Fl-160 antibodies react with *T. cruzi* lysate as expected, but also crossreact with nerve and brain lysates. Anti-Fl-160 antibodies do not react with cardiac lysates. Anti-GST antibodies do not react with *T. cruzi*, or any of the tissue lysates.

By immunoperoxidase staining, we were able to confirm and localize this crossreactivity to processes in nerve and brain (Fig. 5). The processes seen in brain were in highest density in white matter. The same pattern of staining was seen by immunofluorescence. Double immunofluorescent staining with mouse mAbs to 68- and 130-kD neurofilaments and rabbit antibodies to Fl-160 reveals similar localiza-
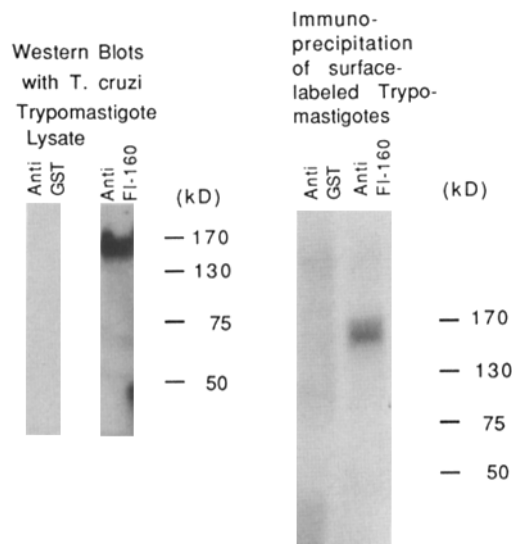

FIGURE 3. Autoradiogram of Western blot and immunoprecipitation reveals trypomastigote Fl-160 protein. The two left lanes are 50 μg/lane of trypomastigote lysate on 10% polyacrylamide SDS gel, Western blotted to nitrocellulose, and reacted with 2 μg/ml anti-GST or anti-Fl-160 antibodies. The two right lanes are surface-^{125}I-labeled trypomastigote lysates immunoprecipitated with anti-GST or anti-Fl-160 antibodies and run on a 4–12% gradient polyacrylamide SDS gel.
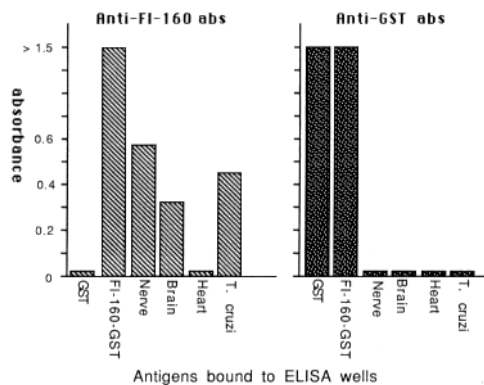
FIGURE 4. ELISA shows anti-Fl-160 antibody crossreactivity with nerve and brain, but not heart tissue. Bars represent absorbance found with each tissue bound to ELISA microwells, reacted with 2 μg/ml rabbit anti-Fl-160 or anti-GST antibodies, and developed with avidin-biotin-peroxidase-ABTS system.

tion in nerve and brain, though some processes in brain stained with anti-Fl-160 antibodies appear not to stain with antineurofilament antibodies (not shown). Human sciatic nerve also stained with anti-Fl-160 antibodies, but not anti-GST antibodies (not shown). No staining by anti-Fl-160 antibodies was observed on skeletal muscle, liver, kidney, or cardiac tissues.
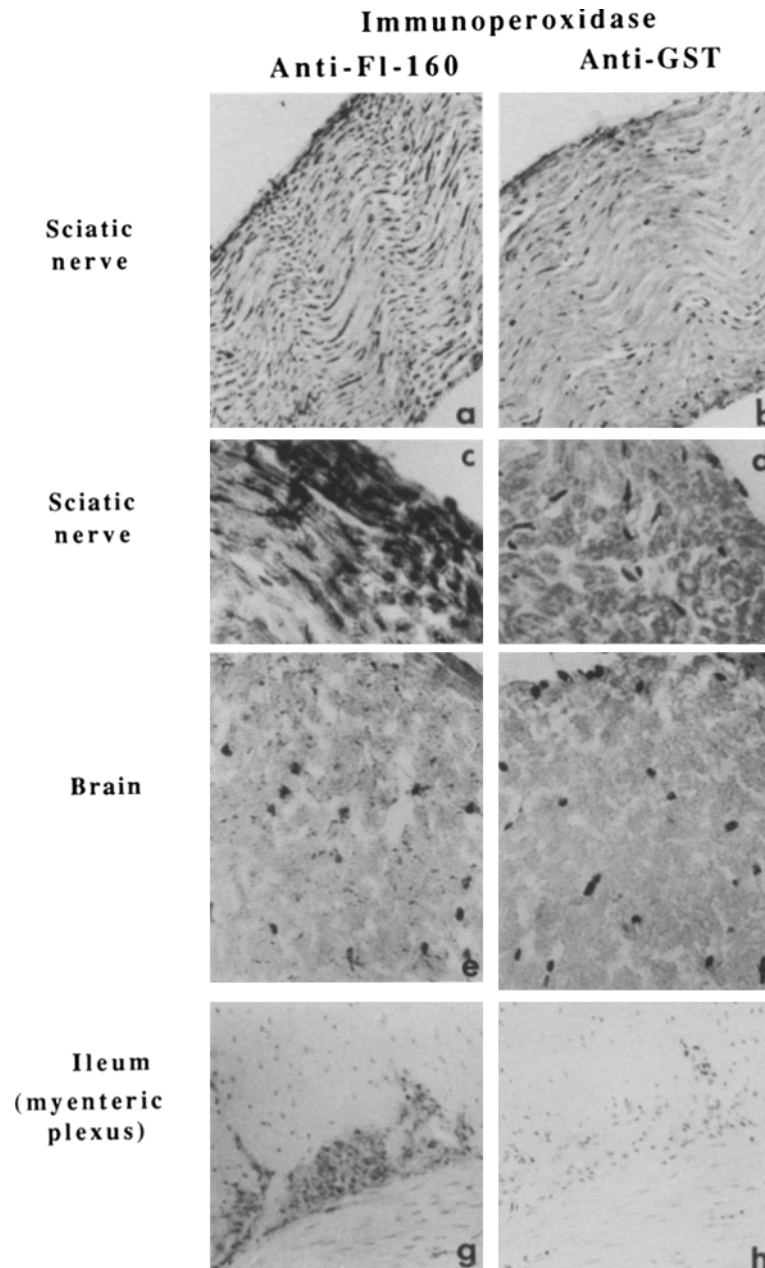
Immunoperoxidase staining revealed anti-Fl-160 antibodies react with the myenteric plexus of neurons between the smooth muscle layers of mouse ileum (Fig. 5). This is particularly interesting because the myenteric plexus is destroyed in chronic disease, leading to the characteristic pathology of megaesophagus and megacolon.

The antibodies recognizing determinants in nervous tissues were confirmed to be the same as those reacting with *T. cruzi* by absorption studies. Absorption of 0.3 μg/ml anti-Fl-160 antibodies with 50 μg/ml nerve extract (but not cardiac extract) abrogated immunofluorescence of trypomastigotes and absorption of 0.3 μg/ml anti-Fl-160 with 50 μg/ml trypomastigote extract (but not epimastigote extract) abrogated immunofluorescence with sciatic nerve (not shown).

Anti-Fl-160 Antibodies Recognize a 48-kD Protein in Brain and Sciatic Nerve Extracts. Western blots and immunoprecipitation demonstrate the molecular mass of the cross-reactive nervous tissue protein recognized by anti-Fl-160 antibodies. A 48-kD band was seen on Western blots of brain extracts exposed to anti-Fl-160 (Fig. 6). This band was not seen in liver, kidney, or heart extracts. A 48-kD band was also seen by immunoprecipitation of ^{125}I-labeled sciatic nerve extract with anti-Fl-160 antibody (not shown). This band was not seen with control immunoprecipitations with anti-GST antibody and labeled nerve extract, or with anti-Fl-160 antibody and labeled cardiac extract. Thus, a nervous tissue-specific 48-kD protein is recognized by anti-Fl-160 antibodies.

Molecular Expression of Fl-160 RNA. We analyzed the gFl-160 insert to determine what part of it is transcribed and translated. The gFl-160 DNA insert is 5 kb and the restriction map is shown in Fig. 7. Based on the 60-kD size of the gFl-160-encoded contribution to the fusion proteins (see first section of Results) and assuming an average molecular mass of 110 daltons per amino acid, ~550 amino acids are expressed from the Fl-160 insert. This means that 1,650 bp are expressed, or about one-third of the total Fl-160 insert.

Northern analysis revealed the expression of gFl-160-hybridizing RNA to be differen-

Immunoperoxidase

Anti-Fl-160   Anti-GST

FIGURE 5. Immunoperoxidase staining with anti-Fl-160 antibodies demonstrates crossreactivity with processes of nerve and brain and the myenteric plexi of gut. *a, c, e,* and *g* are exposed to 2 μg/ml anti-Fl-160 antibodies and *b, d, f,* and *h* are exposed to 2 μg/ml anti-GST. All are developed with avidin-biotin-peroxidase system and counterstained with hematoxylin. *a* and *b* show mouse sciatic nerve in longitudinal section, where anti-Fl-160 reactivity is seen to run longitudinally along the processes of the nerve. *c* and *d* are high-power magnifications of mouse sciatic nerve in partial crosssection (*lower right*) showing anti-Fl-160 antibodies reactivity underneath the myelin sheath, within the nerve processes. *e* and *f* show reactivity of anti-Fl-160 antibodies with many processes in mouse brain, most cut in cross section, but occasionally seen longitudinally for a short distance. *g* and *h* show anti-Fl-160 antibodies react with myenteric plexi of neurons, found between two perpendicularly oriented smooth muscle layer in mouse ileum.
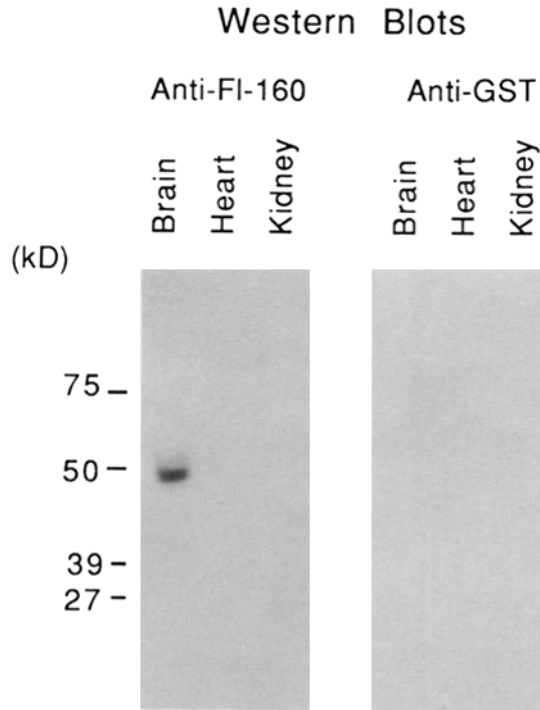
Western Blots

FIGURE 6.   Autoradiograms of Western blots of brain extract demonstrate anti-Fl-160 antibodies crossreact with a 48-kD protein. 100 μg/lane of tissue extracts from brain, heart, and kidney were run on 10% polyacrylamide SDS gels, transferred to nitrocellulose, and exposed to 2 μg/ml affinity-pure rabbit anti-Fl-160 or anti-GST antibodies.
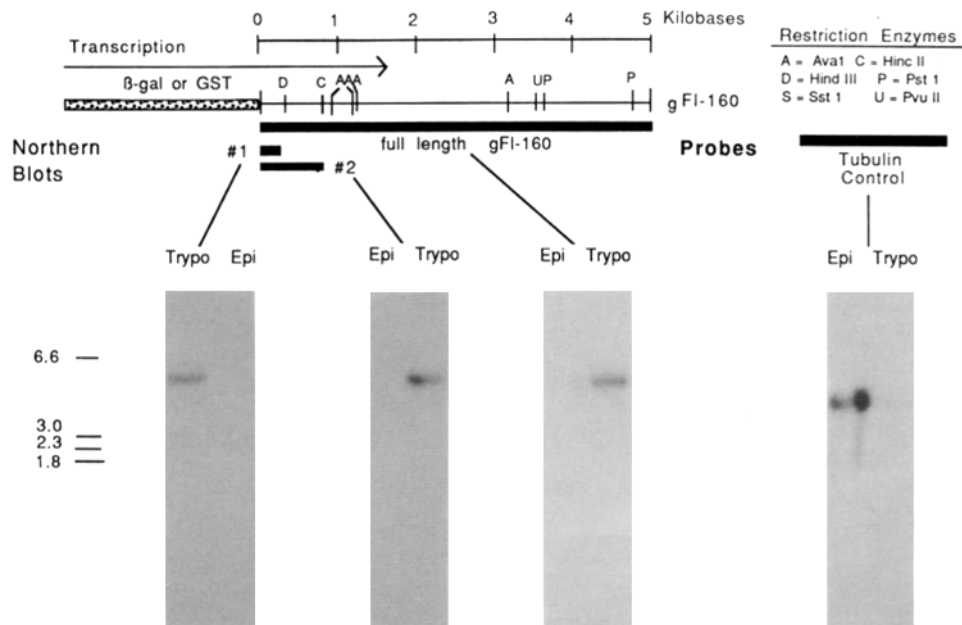

FIGURE 7.   Restriction map of gFl-160 DNA and Northern Analysis of gFl-160 fragments. The restriction enzyme map of gFl-160 is shown with the transcriptional direction in the recombinant expression vectors. The probes, shown as dark bands, were hybridized to Northern blotted RNA from epimastigotes (Epi, at 3 μg/ln) and trypomastigotes (Trypo, at 1 μg/ln). A probe of T. brucei tubulin cDNA acts as a control to show the epimastigote RNA is intact.

tiation specific. The gFl-160 probe hybridizes to a single 4.5-kb band present in trypomastigote RNA, but not epimastigote RNA (Fig. 7). This is consistent with the finding that anti-Fl-160 antibody reacts only with trypomastigotes, and not epimastigotes or amastigotes. Control tubulin probe hybridizes to both trypomastigote and epimastigote RNA, demonstrating the epimastigote RNA to be intact. In experiments not shown, we have found the 4.5-kb gFl-160-hybridizing RNA to be in the poly(A)$^+$ fraction, consistent with it being mRNA. Thus, the absence of Fl-160 antigen of epimastigotes is likely due to transcriptional regulation. That amastigotes express gFl-160-related messages cannot be excluded, given that trypomastigote RNA is contaminated with amastigote RNA. Nonetheless, the lack of epimastigote antigen expression correlating with lack of transcription, and lack of amastigote antigen expression, suggests that the gFl-160-hybridizing mRNA is expressed in a differentiation-specific manner solely in the trypomastigote form.

The finding that gFl-160 DNA hybridizes to a 4.5-kb RNA is in keeping with the 160-kD molecular mass of the trypomastigote protein. An mRNA of that size would be able to code for a protein of at least 166 kD.

Discussion

We have shown that a *T. cruzi* DNA clone (gFl-160) expresses a recombinant protein that corresponds to a 160-kD flagellum-associated surface protein of trypomastigotes that is recognized by sera from chronic infection. Furthermore, antibodies directed to epitopes on this recombinant protein crossreact with nervous tissue in the central nervous system, peripheral nerve, and myenteric plexi, and in the former two tissues, react with a 48-kD protein. The identity of this protein is unknown, but is under current investigation.

Chronic *T. cruzi* infection can lead to nervous tissue pathology, most typified by megaesophagus and megacolon disease, caused by the destruction of myenteric plexi in the gut. Autoimmune processes have been hypothesized to cause this pathology in chronic *T. cruzi* infection. *T. cruzi* antigens that antigenically mimic mammalian nervous tissue antigens have been implicated in the generation of autoimmunity by molecular mimicry. The best data supporting this hypothesis is the *T. cruzi*-reactive T cell lines, which also proliferate with nervous tissue antigens, can passively transfer pathologic damage to naive mice (12, 13). Since the antigens used to generate these T cell lines were not well defined, it is difficult to study the generation of this autoimmune response. Fl-160 is a protein that is a candidate antigen for this autoimmune response. We have had difficulty immunizing mice with this antigen to obtain a serologic response, despite the use of adjuvants and multiple immunizations. This raises two points. First, the parasite may use antigenic mimicry to help blunt the immune response. Second, it seems likely that to generate autoimmunity, both antigenic mimicry plus breakdown of immune regulation preventing response to self-antigens are needed. This breakdown of immune regulation may occur during the acute infection, where massive polyclonal lymphocyte proliferation has been documented (22). A similar massive polyclonal lymphocyte proliferation has been correlated with the generation of autoimmune responses in genetically susceptible mice (23, 24).

Once the autoimmune response to Fl-160 is established, perhaps the lack of myelination of the myenteric plexi, exposing their antigens to circulating immune cells, explains the particular sensitivity of myenteric plexi to immune destruction in chronic

Chagas' disease. We are beginning to study the cellular immune response to the Fl-160 antigen to help determine its role in pathology.

Several findings extend our results to human disease. First, the anti-Fl-160 antibodies bind to human sciatic nerve, confirming this crossreactive antigen is present in human tissues. Second, humans make antibody to the Fl-160 antigen when infected. In preliminary experiments, we have found that approximately one-third of *T. cruzi*-infected individuals have demonstrable antibody to Fl-160. ELISA titers of 1:1,600 have been detected in some chronically infected individuals. Geographically matched uninfected controls do not have antibodies to Fl-160. Thus, humans are induced by infection to recognize this crossreactive antigen. We are now collecting enough specimens to attempt to correlate reactivity to Fl-160 with disease state.

The Fl-160 protein is limited to the trypomastigote form of *T. cruzi* as evidenced by the fact that (*a*) Northern analysis shows hybridization of Fl-160 DNA to trypomastigote RNA, but not epimastigote RNA; (*b*) immunofluorescence with anti-Fl-160 antibodies shows reactivity with trypomastigotes, but not epimastigotes or amastigotes; and (*c*) Western analysis shows reactivity with trypomastigote lysates, but not epimastigote lysates. These findings are conditional on the fact that most of our trypomastigote preparations were contaminated with amastigotes. Enrichment of trypomastigotes from amastigotes by percoll gradient centrifugation led to enrichment of anti-Fl-160 Western blot reactivity of the trypomastigote lysates and marked diminution of reactivity of amastigote lysates (not shown). Furthermore, the amastigotes were morphologically distinguishable by immunofluorescence and did not fluoresce with anti-Fl-160. Antibodies to another recombinant protein stained amastigotes, and not trypomastigotes, thus, staining the parasites in these preparations not stained with anti-Fl-160 (not shown). Thus, it is likely that the Fl-160 protein is expressed in a differentiation-specific manner, by transcriptional control, on trypomastigotes.

That anti-Fl-160 antibodies stained along the flagellum is particularly interesting, given that the epimastigote flagellum was not stained. This suggests that the membrane overlying the flagellum of the mammalian form is distinct from that of the insect form. The expression of Fl-160 exclusively in the mammalian form may reflect a role for specific interaction with mammalian cells.

Also of note is the strong anti-Fl-160 immunofluorescence seen on the 3T3 cells that are infected with *T. cruzi*. This suggests that the antigen can be recognized by the immune system on the surface of infected cells. The antigen was found on 3T3 cells in infected cultures that had no identifiable intracellular parasites, but not on 3T3 cells in uninfected cultures. This could mean the Fl-160 antigen is absorbed onto uninfected cells, as has been shown with extracts of *T. cruzi* antigens (25). The source of the absorbed Fl-160 antigen could be from antigen shed into the medium, or after direct interaction with trypomastigotes. As proposed by Hudson and co-workers (25), the absorption of *T. cruzi* antigens to uninfected cells may lead to damage of uninfected cells by the immune response directed to *T. cruzi* antigens.

Summary

Chagas' disease, caused by *Trypanosoma cruzi*, is an excellent model for autoimmune disease induced by an infectious agent. Transfer of T cells, directed against crossreactive antigens of *T. cruzi* and nervous tissue, have been shown to reproduce

pathology found in chronic Chagas' disease. We used recombinant DNA technology to characterize one of these crossreactive antigens (Fl-160). We have cloned DNA from *T. cruzi*, which expresses a protein corresponding to a 160-kD protein found on the surface of the trypanosome, overlying the flagellum. This clone hybridizes to a 4.5-kb poly(A)$^+$ RNA that is distributed in a differentiation-specific manner, suggesting expression of this protein is transcriptionally controlled. Antibodies to this protein crossreact with a 48-kD mammalian nervous tissue protein found in sciatic nerve, brain, and myenteric plexi of gut. The myenteric plexi are destroyed by inflammatory infiltrates in Chagas' disease, leading to the characteristic megaesophagus and megacolon Chagas' disease pathology. Thus, this antigen is a candidate antigen for autoimmune mimicry leading to nervous tissue pathology.

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