The Gene for a T Lymphocyte Triggering Factor from African Trypanosomes

By Tushar Vaidya,* Moiz Bakhiet,‡§ Kent L. Hill,* Tomas Olsson,‡ Krister Kristensson,‖ and John E. Donelson*

*From the *Department of Biochemistry, University of Iowa, and the Howard Hughes Medical Institute, Iowa City, Iowa 52242; ‡Department of Medicine, Unit for Molecular Medicine, Karolinska Hospital, Stockholm, Sweden; §Division of Neurology, Huddinge University Hospital, Karolinska Institute, Stockholm, Sweden; and the ‖Department of Neuroscience (Division of Neurodegenerative Disease Research), Karolinska Institute, Stockholm, Sweden*

Summary

An early and essential event in the protective immune response against most viruses and protozoa is the production of interferon-γ (IFN-γ). In contrast, during infection with African trypanosomes, protozoan parasites that cause human sleeping sickness, the increased levels of IFN-γ do not correlate with a protective response. We showed previously that African trypanosomes express a protein called T lymphocyte triggering factor (TLTF), which triggers $CD8^+$ T lymphocytes to proliferate and to secrete IFN-γ. Here, we isolate the gene for TLTF and demonstrate that the recombinant version of TLTF specifically induces $CD8^+$, but not $CD4^+$, T cells to secrete IFN-γ. Studies with TLTF fused to the green fluorescent protein show that TLTF is localized to small vesicles that are found primarily at or near the flagellar pocket, the site of secretion in trypanosomes. TLTF is likely to be only the first example of a class of proteins that we designate as trypanokines, i.e., factors secreted by trypanosomes that modulate the cytokine network of the host immune system for the benefit of the parasite.

African trypanosomiasis is a widespread, fatal disease in Africa that is commonly called sleeping sickness in humans and ngana in cattle. These diseases are caused by several different species or subspecies of *Trypanosoma*, which are transmitted by tsetse flies to the bloodstream of their mammalian hosts where they circulate extracellularly and can eventually affect the central nervous system. Mortality occurs from either massive parasitosis or secondary infections due to immunosuppression, an important trait of the disease.

Previously, we showed that trypanosomes release a T lymphocyte triggering factor (TLTF)[1] that induces $CD8^+$ T cells to secrete IFN-γ (1, 2); subsequent studies indicated that TLTF binds to the CD8 molecule on T cells (3). When rodents are infected with *Trypanosoma brucei*, IFN-γ production in the spleen increases markedly. Moreover, in rats depleted of $CD8^+$ T cells by injection of anti-CD8 monoclonal antibody or in knockout mice carrying a deletion of the CD8 gene, a trypanosome infection does not induce as much IFN-γ production, the parasitemia is decreased and the infected animals survive longer (3). In passive immunotherapy experiments, a mouse monoclonal antibody directed against TLTF greatly reduces parasite levels in, and increases survival of, animals infected with *T. brucei* (4, 5). Here, we identify the gene for TLTF and show that bacterially expressed TLTF has T cell stimulatory activity that is inhibited by monoclonal antibodies raised against the native protein. We further show that TLTF has sequence similarity with a mammalian protein and that the subcellular localization of a green fluorescent protein (GFP)–TLTF fusion protein is consistent with that expected for a secreted protein.

[1]*Abbreviations used in this paper:* ADF, adult T cell leukemia-derived factor; GFP, green fluorescent protein; GST, glutathione-S-transferase; IGIF, interferon-γ–inducing factor; THIO, thioredoxin; TLTF, T lymphocyte triggering factor.

Materials and Methods

Molecular Biology Procedures. The TLTF cDNA was identified by immunoscreening a λZapII cDNA library of bloodstream *T. b. rhodesiense* RNA (6) with rabbit antiserum against affinity-purified TLTF (4) at a 1:2000 dilution. The antiserum was preadsorbed with *Escherichia coli* lysate before use. The secondary antibody was a horseradish peroxidase–linked donkey anti–rabbit IgG (Amersham Corp., Arlington Heights, IL) used at a 1:5000 dilution. At all stages, a 5% solution of nonfat powdered milk was used to block nonspecific binding. Potentially positive plaques were detected using the enhanced chemiluminescent system (Amersham Corp., Arlington Heights, IL) and taken through two more rounds of screening. The cDNA sequence was determined by standard sequencing procedures using the Sequenase 2.0 kit (US Biochemicals,

J. Exp. Med. © The Rockefeller University Press • 0022-1007/97/08/433/06 $2.00
Volume 186, Number 3, August 4, 1997 433–438
http://www.jem.org

Cleveland, OH). Homology searches of databases were performed using the BLAST algorithm (7). Protein sequence alignments were done using the University of Wisconsin GCG software package (8).

The recombinant thioredoxin (THIO) and glutathione-S-transferase (GST) fusion proteins were generated according to protocols provided by the supplier of the expression plasmids (AMRAD, Australia; Invitrogen, California). The PCR amplified fragments of the TLTF cDNA that were cloned into the expression plasmids contained the TLTF start codon and either the complete TLTF open reading frame or the first 145 codons. Bacterial lysates were fractionated by SDS-PAGE to resolve the fusion proteins. Rabbits and mice were immunized with crushed gel slices containing the fusion protein (THIO–TLTF) or with fusion protein that had been further purified by electroelution and dialysis (GST–TLTF) according to standard procedures (9). Plasmid DNA containing the gene for GST–OV7 was the gift of Dr. J. Catmull (University of Iowa, Iowa City, IA).

The *T. brucei* expression vector pHD–GFPm3 was generated by replacing the luciferase gene of pHD1 (10) with the GFPmut3 gene (11). This mutant version of GFP gives 30- to 100-fold greater fluorescence than wild-type GFP from *Aquorea victoria*. To generate the GFP–TLTF fusion construct, EcoRI sites were introduced in front of the GFP stop codon and the TLTF start codon via PCR amplification of the corresponding genes using GFP- or TLTF-specific primers, which included an EcoRI restriction site. In the case of TLTF, we used the same approach to introduce a BamHI site immediately following the TLTF stop codon. The TLTF open reading frame was then cloned in frame behind the GFP gene to generate pHD–GFPm3–TLTF.

DNA Transfections. Procyclic trypanosomes (clone YTAT1.1, obtained from E. Ullu, Yale University, New Haven, CT) were maintained in Cunningham's SM medium (12) supplemented with 20% FCS. Cells were harvested from mid-log phase cultures ($2–4 \times 10^6$ cells/ml), washed once with electroporation medium (120 mM KCl, 0.15 mM $CaCl_2$, 10 mM K_2HPO_4, 25 mM Hepes, 2 mM EDTA, 5 mM $MgCl_2$, pH 7.6) and resuspended in the same buffer at $1–3 \times 10^8$ cells/ml. DNA (50 μg) was introduced into cells by electroporation with a BioRad Gene Pulser using two pulses of 1,500 V at 25 μF in 0.4 cm cuvettes. Transfected cells were transferred to fresh culture medium and examined for GFP expression 16–24 h after transfection. For ethidium bromide staining, cells were incubated with 2 mg/ml ethidium bromide for 15 min, then washed twice with PBS. Cells were examined using a BioRad MRC-1024 Laser Scanning Confocal Microscope.

IFN-γ Assays. Purification of native TLTF, isolation of mononuclear cells and the spot assay for IFN-γ secretion were performed as described previously (1, 3, 4). The final concentration of concanavalin A in the assays was 5 μg/ml. The final concentration of monoclonal antibody MO1 was 5 μg/ml. All assays were conducted using cells from $CD4^-CD8^+$ mice except where indicated. The origin of the $CD4^-CD8^+$ and $CD4^+CD8^-$ mice were described earlier (13). Most of the assays for IFN-γ secretion that involved the recombinant fusion proteins were conducted blind using protein prepared in the United States and sent in coded tubes to Sweden, and were done at least in triplicate.

Results and Discussion

A monoclonal antibody, called MO1, was used to affinity purify TLTF from bloodstream trypanosome extracts. Monovalent polyspecific antiserum directed against the affinity-purified protein was generated in rabbits and used to immunoscreen a cDNA expression library of total RNA isolated from bloodstream forms of *T. b. rhodesiense*, a subspecies that causes the human disease (6). A cDNA clone was identified that remained immunopositive through multiple rounds of screening. The nucleotide sequence of this cDNA revealed that it was a partial length clone lacking the 39 nucleotide sequence of the 5′ spliced leader, a universal feature of trypanosome mRNAs. A full-length coding sequence was obtained from the product of a reverse transcriptase-PCR amplification of total trypanosome RNA using an internal 3′ primer derived from the TLTF partial cDNA sequence and a 5′ primer matching the 5′ spliced leader sequence. Fig. 1 shows the deduced 453-amino acid sequence of the encoded protein, a 54-kD hydrophilic polypeptide.

The amino acid sequence of TLTF bears no obvious similarity to IGIF (IFN-γ–inducing factor), a protein recently identified in mouse liver that induces IFN-γ production by $CD4^+$ T lymphocytes (14). The lack of similarity between these two proteins may be because IGIF is thought to target $CD4^+$ cells, whereas TLTF interacts specifically with the CD8 molecule. However, computer searches did identify similarities to two other sequences that have been deposited in GenBank without further characterization. One encodes a mouse protein of 489 residues designated as a growth arrest–specific protein. The other protein is encoded by a randomly sequenced human cDNA

Figure 1. Predicted amino acid sequence of TLTF and comparison to a mouse growth arrest–specific protein. The trypanosome cDNA sequence predicts a hydrophilic, 453-amino acid protein of 54 kD. In the alignment with the mouse protein (GenBank accession No. U19589) similar amino acid residues, including conservative substitutions, are outlined and positions of identity are shaded. The two proteins exhibit 59% amino acid similarity and 35% identity. The deduced protein encoded by a human expressed sequence tag (dbEST accession No. W21172) is nearly identical (87%) to the mouse protein and also displays 34% identity with TLTF (data not shown). The trypanosome TLTF sequence data are available from EMBL/GenBank/DDBJ under accession number AF012853.

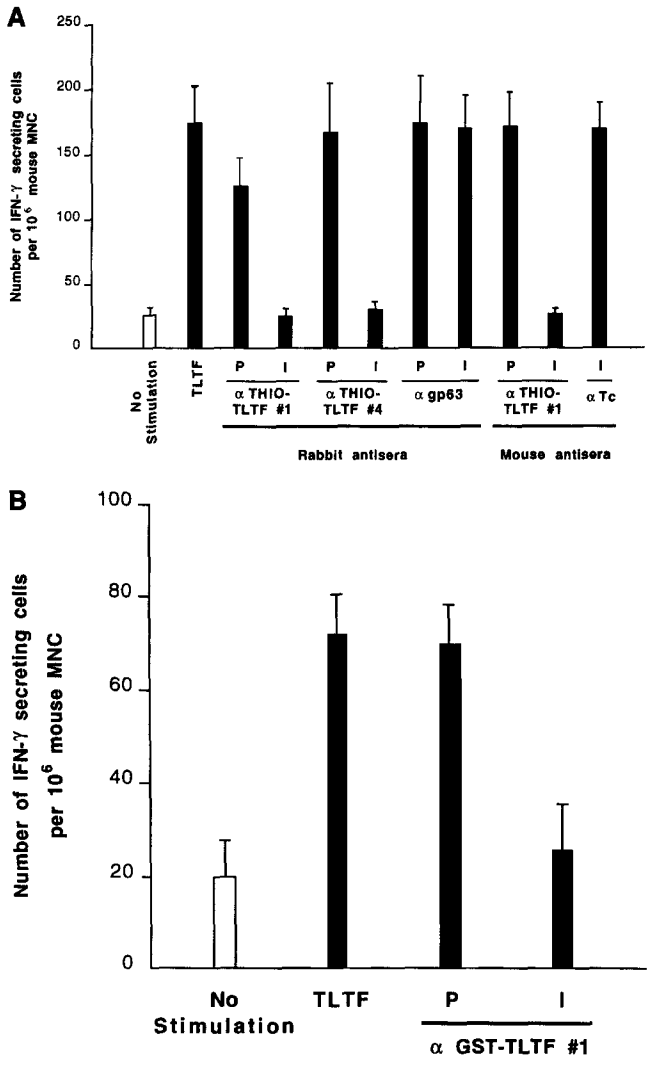

Figure 2. Antisera directed against the two different forms of recombinant TLTF inhibit interfere with the activity of native TLTF. (*A*) Results obtained with antisera raised against THIO–TLTF. *P* and *I* denote preimmune and immune serum, respectively. α-THIO–TLTF #1 is serum directed against a fusion protein containing the entire TLTF molecule. α-THIO–TLTF #4 is serum directed against a fusion protein containing only the NH_2-terminal 145 amino acids of TLTF. α-gp63 is serum directed against an unrelated *Leishmania chagasi* protein, gp63 (30); α-Tc is serum directed against a recombinant version of an unrelated *Trypanosoma cruzi* antigen, gp72 (31) (the gift of K. Otsu and L.V. Kirchhoff, University of Iowa, Iowa City, IA). The dilution of serum used in each case was 1:5,000. (*B*) Results obtained with α-GST–TLTF #1, a rabbit antiserum directed against a GST fusion protein containing the entire TLTF protein (1:1,000 dilution).

from a fetal lung cDNA library. Comparison of these proteins with TLTF reveals several regions of high sequence identity (Fig. 1). Although the properties and functions of these two mammalian proteins are yet to be described, their percent identity to TLTF (35% identity, 58% similarity) appears too large to be fortuitous.

The trypanosome cDNA was cloned into two different bacterial expression systems in which the NH_2-terminal fusion partner is either GST or THIO. In each case, a PCR-derived fragment of the TLTF cDNA was cloned such that the methionine start codon is in frame with the last amino acid codon in the GST or THIO gene. In the THIO case, an additional clone was constructed in which the TLTF segment terminates at codon 145. The recombinant proteins were used to generate polyclonal sera in rabbits and mice. Each serum was then tested for its inhibition of the ability of native TLTF to induce T cells to secrete IFN-γ. Fig. 2 *A* shows that rabbit antisera directed against either of the two versions of THIO–TLTF (#1 and #4) reduced the activity of native TLTF to the background levels seen in the absence of TLTF. Similarly, mouse antiserum raised against a separate aliquot of THIO–TLTF #1 also reduced the activity of native TLTF to background levels. The presence of the corresponding preimmune serum did not significantly diminish TLTF activity. In control experiments, rabbit antiserum against *Leishmania chagasi* gp63 and mouse antiserum against *Trypanosoma cruzi* gp72 (each of which is a surface protein of its respective parasite) had no effect on the biological activity of native TLTF. Fig. 2 *B* shows that similar results were obtained in parallel experiments using rabbit antiserum directed against the GST–TLTF fusion protein.

The THIO and GST fusion proteins were also tested for TLTF activity, i.e., the ability to induce T cells to secrete IFN-γ. Fig. 3 *A* shows that GST–TLTF induces IFN-γ secretion from $CD8^+$, but not $CD4^+$, T cells. The splenocytes used in these assays were obtained from gene knockout mice that lacked either the CD4 gene or the CD8 gene (13). Similar to native TLTF, the GST–TLTF fusion protein induced IFN-γ secretion only from the population containing $CD8^+$ cells and not from the cells derived from CD8 knockout mice. Control experiments in which concanavalin A was used as a mitogen demonstrated that both populations of cells were intrinsically capable of secreting IFN-γ in response to an appropriate signal. Fig. 3 *B* demonstrates in a separate series of experiments that the ability of GST–TLTF to induce IFN-γ secretion is specifically due to the TLTF portion of the molecule. Neither GST alone nor another unrelated GST fusion protein, GST–OV7, possessed an activity significantly greater than the background level. In similar experiments not shown, THIO–TLTF, also possessed a similar level of TLTF activity.

Next, we examined whether antibodies that specifically recognize the native TLTF molecule can inhibit the activity of recombinant TLTF. We previously demonstrated that the monoclonal antibody MO1 reduces the ability of native TLTF to induce IFN-γ secretion from $CD8^+$ cells (4). Fig. 4 shows the results obtained when the activity of GST–TLTF was measured in the presence of MO1. Under the conditions of the assay, the activity of native TLTF was reduced by 62% in the presence of MO1. Consistent with the interpretation that GST–TLTF is the recombinant version of this protein, a concomitant decrease of 60% in the activity of GST–TLTF occurred in the presence of MO1. In the case of the unrelated GST–OV7, the number of IFN-γ–secreting cells detected in the assay was unaffected by the presence or absence of MO1.

We previously showed that TLTF is released as a soluble

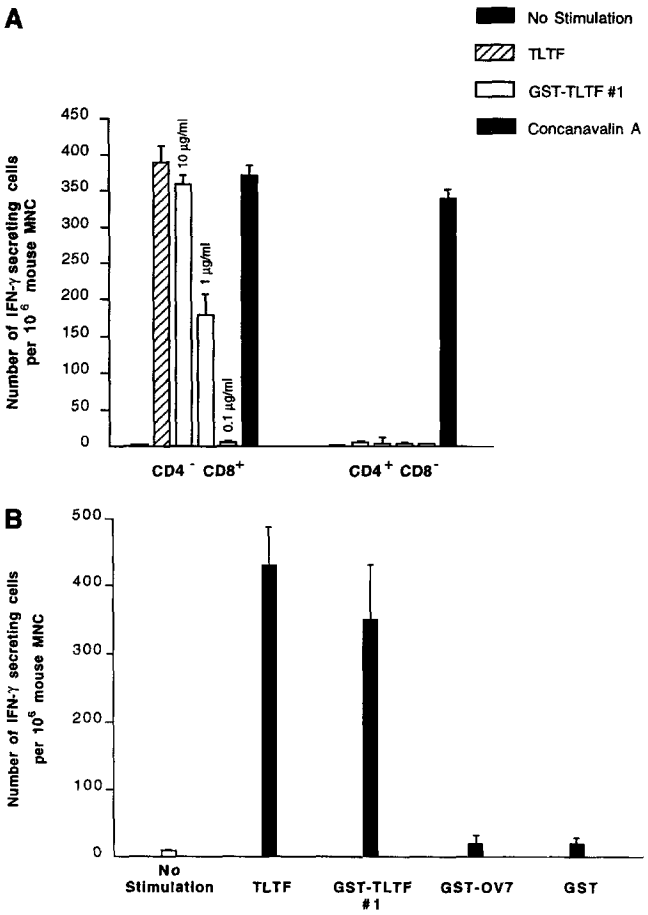

Figure 3. Recombinant TLTF has biological activity. (*A*) Results demonstrating the specificity of GST–TLTF #1 activity on mononuclear cells from $CD4^-CD8^+$ and $CD4^+CD8^-$ mice. The concentrations of GST–TLTF #1 used in the reactions are indicated. (*B*) Comparison of the activities of native TLTF, GST–TLTF #1, GST–OV7, and GST alone. GST–OV7 is an unrelated GST fusion protein prepared in the same way as GST–TLTF. Assays in B were done using cells from $CD4^-CD8^+$ mice. The concentration of the recombinant proteins and GST in the assays shown in *A* and *B* was 10 μg/ml. Conconavalin A was used at a final concentration of 5 μg/ml.

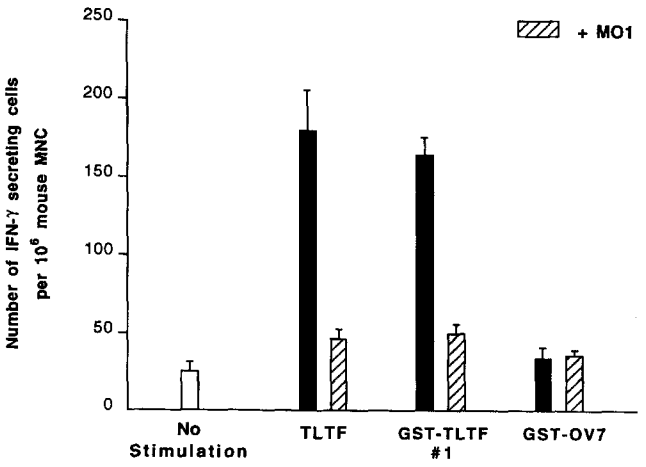

Figure 4. Monoclonal antibody MO1 directed against native TLTF inhibits the biological activity of both native TLTF and recombinant GST–TLTF. The IFN-γ assays were conducted in the absence (*closed bars*) or presence (*cross-hatched bars*) of MO1.

factor from *T. brucei* into the culture medium (15) and yet the amino acid sequence of TLTF (see Fig. 1) does not contain a classical NH_2-terminal signal sequence similar to those found in most secreted eukaryotic proteins (16). Because secretion of TLTF is likely essential for its immunomodulatory function, we investigated how it is targeted to the exterior of the cell. This is an especially relevant question in trypanosomatids, because the entire surface of these organisms is covered by a very dense protein coat that restricts possible sites of endocytosis and secretion to the flagellar pocket (17). This organelle derives from a specialized invagination of the cell membrane that forms at the site where the flagellum emerges from the cell and is relatively protected from the assaults of the immune system of the host. The kinetoplast of *T. brucei* is also located near the base of the flagellum and serves as a marker for the subcellular location of the flagellar pocket.

To examine the cellular fate of TLTF, we fused it to GFP from *Aquorea victoria* (11). Constructs encoding either this fusion protein, or GFP alone, were expressed in transiently transfected trypanosomes, which were examined by laser scanning confocal microscopy (Fig. 5). Because Northern blot analysis indicated that TLTF is expressed in both procyclic and bloodstream form trypanosomes (data not shown), we used the more readily cultured procyclic forms for these transfection experiments. Western blots using an α-GFP polyclonal antiserum (Clonetech, Palo Alto, CA) confirmed that transfected trypanosomes were producing a protein of the expected size (data not shown). Cells in the bottom panel of Fig. 5 were also stained with ethidium bromide to visualize the nucleus and kinetoplast (red staining). As can be seen in the top panel of Fig. 5, GFP alone is expressed evenly throughout the entire cell. The GFP–TLTF fusion protein, on the other hand, is restricted to vesicles that are usually, but not exclusively, located at or near the trypanosome flagellar pocket (Fig. 5, *bottom*). These results are consistent with TLTF being a secreted protein, as shown previously (15), and demonstrate that signals for TLTF targeting are located within the protein itself, even though it does not contain a hydrophobic NH_2-terminal signal sequence. Thus, TLTF is added to the growing list of secreted eukaryotic proteins for which the amino acid sequence gives no indication of a readily identifiable targeting signal (18). Interestingly, many such proteins play a role in immune system function (e.g., IL-1β and adult T cell leukemia-derived factor [ADF]. IL-1β is a monocyte-derived, proinflammatory cytokine that stimulates a broad range of cell types. ADF mediates upregulation of IL-2 receptor expression and may be involved in the abnormal proliferation of T cells observed in some types of leukemogenesis (19). Although some features of export may be shared between leaderless secreted proteins, the mechanisms responsible are unknown. Recent data indicate that multiple mechanisms exist (20) and that two dif-

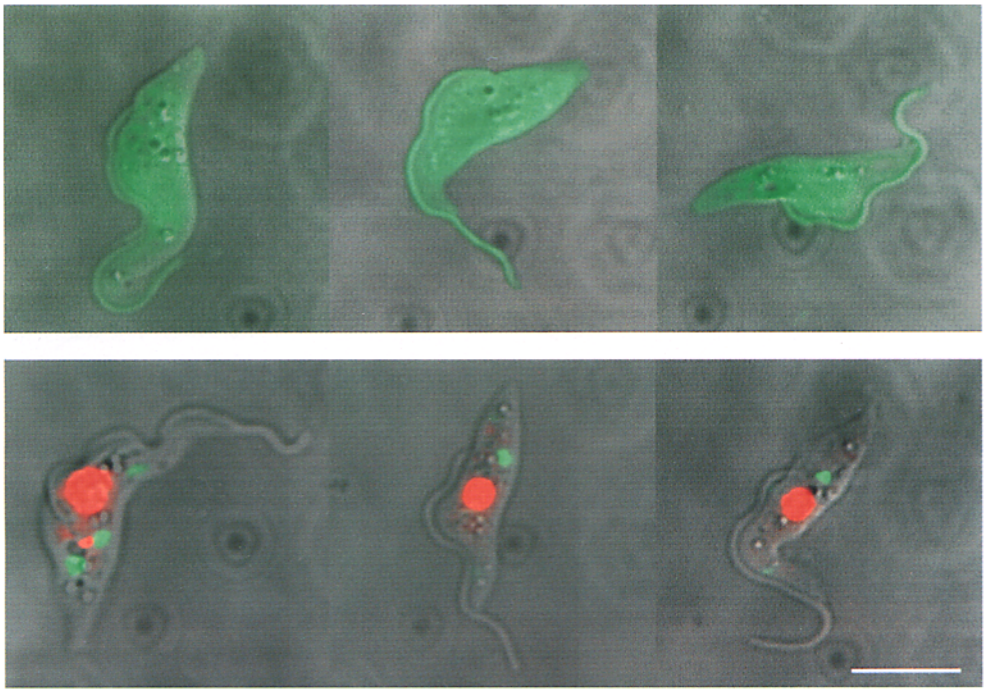

Figure 5. TLTF targeting signals direct a heterologous reporter protein (*GFP*) to vesicles that are localized to the trypanosome flagellar pocket. Procyclic *T. brucei* cells were transiently transfected with a *T. brucei* expression plasmid containing the GFP gene alone (*top*) or a plasmid encoding a GFP–TLTF fusion protein (*bottom*). The transfected cells were examined by laser scanning confocal microscopy and the transmitted and fluorescent images were superimposed on one another. GFP fluorescence is green. Cells in the bottom panel were additionally stained with ethidium bromide to visualize the nucleus and kinetoplast (*red staining*). The white bar corresponds to 10 μm.

ferent proteins may utilize partially, but not completely, overlapping pathways (21).

TLTF is likely to be the first confirmed member of a cabal of trypanosome-derived, immunomodulatory factors for which we suggest the name trypanokines. Trypanosomal cysteine protease, which is found in the serum of infected animals (22–24), is another potential trypanokine, because cysteine proteases of other microorganisms have been shown to modulate cytokine activity either directly or indirectly (25, 26). Still other unknown mitogenic trypanosome molecules have been proposed previously to be the cause of the massive nonspecific polyclonal activation of B cells during acute trypanosomiasis (27). Parallels have already been drawn between gene rearrangements that generate the antibody repertoires in vertebrates and those responsible for the perpetual antigenic variation in trypanosomes (28). Trypanokines represent another example of the ability of the trypanosome to mimic a normal host function; in this case, the synthesis of cytokines that modulate the immune response. Recent experiments with viruses and infectious bacteria indicate that this strategy may also be commonly used by other microbial pathogens (25, 29). In this regard, the availability of the TLTF gene should facilitate novel approaches towards achieving protection against trypanosomiasis. In addition, recombinant TLTF could find applications in the treatment of other infectious agents, such as *Leishmania* and viruses, for which IFN-γ production is among the first events of a protective response.

We would like to thank D. Russell for conducting preliminary immunogold localization of native TLTF. We are extremely grateful to E. Ullu for the YTAT1.1 trypanosome clone and for her assistance in our efforts to express GFP in *T. brucei*. We would also like to thank B. Cormack and S. Falkow for providing the GFPmut3 cDNA.

This research was funded in part by a grant from the United Nations Development Program/World Bank/World Health Organization Special Program for Research and Training in Tropical Disease to M. Bakhiet, T. Olsson, and K. Kristensson.

Address correspondence to John E. Donelson, Department of Biochemistry, University of Iowa, Iowa City, IA 52242. Phone: 319-335-7889; FAX: 319-335-6764. The current address for Tushar Vaidya is Center for Cellular and Molecular Biology, Uppal Rd., Habshiguda, Hyderbad, India 500007.

Received for publication 9 April 1997 and in revised form 27 May 1997.

References

1. Bakhiet, M., T. Olsson, P. Van der Meide, and K. Kristensson. 1990. Depletion of CD8+ T cells suppresses growth of *Trypanosoma brucei brucei* and interferon-gamma production in infected rats. *Clin. Exp. Immunol.* 81:195–199.
2. Olsson, T., M. Bakhiet, and K. Kristensson. 1992. Interactions between *Trypanosoma brucei* and $CD8^+$ T cells. *Parasitol. Today.* 8:237–239.
3. Olsson, T., M. Bakhiet, B. Hojeberg, A. Ljungdahl, C. Edlund, G. Andersson, H.-P. Ekre, W.-P. Fung-Leung, T. Mak, H. Wigzell, U. Fiszer, and K. Kristensson. 1993. CD8 is critically involved in lymphocyte activation by a *T. brucei brucei*–released molecule. *Cell.* 72:715–727.
4. Bakhiet, M., T. Olsson, C. Edlund, B. Hojeberg, K. Holmberg, J. Lorentzen, and K. Kristensson. 1993. A *trypanosoma brucei brucei*–derived factor that triggers $CD8^+$ lymphocytes to interferon-γ secretion: purification, characterization and protective effects *in vivo* by treatment with a monoclonal antibody against the factor. *Scand. J. Immunol.* 37:165–178.
5. Bakhiet, M., T. Olsson, J. Mhlanga, P. Buscher, N. Lycke, P.H. Van der Meide, and K. Kristensson. 1996. Human and rodent interferon-γ as a growth factor for *Trypanosoma brucei. Eur. J. Immunol.* 26:1359–1364.
6. Alarcon, C.M., H.J. Son, T. Hall, and J.E. Donelson. 1994. A monocistronic transcript for a trypanosome variant surface glycoprotein. *Mol. Cell. Biol.* 14:5579–5591.
7. Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410.
8. Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12:387–395.
9. Harlow, E., and D. Lane. 1988. Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 1–726.
10. Hug, M., V.B. Carruthers, C. Hartmann, D.S. Sherman, G.A.M. Cross, and C. Clayton. 1993. A possible role for the 3′-untranslated region in developmental regulation in *Trypanosoma brucei. Mol. Biochem. Parasitol.* 61:87–96.
11. Cormack, B.P., R.H. Valdivia, and S. Falkow. 1996. FACS-optimized mutants of the green fluorescent protein (GFP). *Gene.* 173:33–38.
12. Cunningham, I. 1977. New culture medium for maintenance of tsetse tissues and growth of trypanosomatids. *J. Protozool.* 24:325–329.
13. Rottenberg, M.E., M. Bakhiet, T. Olsson, K. Kristensson, T. Mak, H. Wigzell, and A. Orn. 1993. Differential susceptibilities of mice genomically deleted of CD4 and CD8 to infections with *Trypanosoma cruzi* or *Trypanosoma brucei. Infect. Immun.* 61:5129–5133.
14. Okamura, H., H. Tsutsul, T. Komatsu, M. Yutsudo, A. Hakura, T. Tanimoto, K. Torigoe, T. Okura, Y. Nukada, K. Hattori et al. 1995. Cloning of a new cytokine that induces IFN-γ production by T cells. *Nature (Lond.).* 378:88–91.
15. Olsson, T., M. Bakhiet, C. Edlund, B. Hojeberg, P.H. Van der Meide, and K. Kristensson. 1991. Bidirectional activating signals between *Trypanosoma brucei* and $CD8^+$ T cells: a trypanosome-released factor triggers interferon-γ production that stimulates parasite growth. *Eur. J. Immunol.* 21:2447–2454.
16. Schatz, G., and B. Dobberstein. 1996. Common principles of protein translocation across membranes. *Science (Wash. DC).* 271:1519–1526.
17. Webster, P., and D.G. Russell. 1993. The flagellar pocket of trypanosomatids. *Parasitol. Today.* 9:201–206.
18. Muesch, A., E. Hartmann, K. Rohde, A. Rubartelli, R. Sitia, and T.A. Rapoport. 1990. A novel pathway for secretory proteins? *TIBS.* 15:86–88.
19. Tagaya, Y., Y. Maeda, A. Mitsui, N. Kondo, H. Matsui, J. Hamuro, N. Brown, K. Arai, T. Yokota, H. Wakasugi, and J. Yodoi. 1989. ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:757–764.
20. Cleves, A., D. Cooper, S. Barondes, and R. Kelly. 1996. A new pathway for protein export in *Saccharomyces cerevisiae. J. Cell Biol.* 133:1017–1026.
21. Rubartelli, A., A. Bajetto, G. Allavena, E. Wollman, and R. Sitia. 1992. Secretion of thioredoxin by normal and neoplastic cells through a leaderless secretory pathway. *J. Biol. Chem.* 267:24161–24164.
22. Gutteridge, W.E. 1987. New antiprotozoal agents. *Int. J. Parasitol.* 17:121–130.
23. McKerrow, J.H. 1993. The proteases and pathogenicity of parasitic protozoa. *Annu. Rev. Microbiol.* 47:821–853.
24. Lonsdale-Eccles, J.D., W.N. Mpimbaza, R.M. Nkhungulu, J. Olobo, L. Smith, O.M. Tosomba, and D.J. Grab. 1995. Trypanosomatid cysteine protease activity may be enhanced by a kininogen-like moiety from host serum. *Biochem. J.* 305: 549–556.
25. Kapur, V., M.W. Majesky, L.-L. Li, R.A. Black, and J.M. Musser. 1993. Cleavage of interleukin 1β (IL-1β) precursor to produce active IL-1β by a conserved extracellular cysteine protease from *Streptococcus pyogenes. Proc. Natl. Acad. Sci. USA.* 90:7676–7680.
26. LaMarre, J., G.K. Wollenberg, S.L. Gonias, and M.A. Hayes. 1991. Cytokine binding and clearance properties of proteinase-activated α2-macroglobulins. *Lab. Invest.* 65:3–14.
27. Sileghem, M., J.N. Flynn, A. Darji, P. De Baetselier, and J. Naessens. 1994. African Trypanosomiasis. *In* Parasite Infections and the Immune System. F. Kierszenbaum, editor. Academic Press, Inc., San Diego. 1–51.
28. Donelson, J.E. 1995. Mechanisms of antigenic variation in *Borrelia hermsii* and African trypanosomes. *J. Biol. Chem.* 270: 7783–7786.
29. Gooding, L.R. 1992. Virus proteins that counteract host immune defenses. *Cell.* 71:5–7.
30. Wilson, M.E., K.K. Hardin, and J.E. Donelson. 1989. Expression of the major surface glycoprotein of *Leishmania donovani chagasi* in virulent and attenuated promastigotes. *J. Immunol.* 143:678–684.
31. de Jesus, A.R., R. Cooper, M. Espinosa, J.E. Gomes, E.S. Garcia, S. Paul, and G.A. Cross. 1993. Gene deletion suggests a role for *Trypanosoma cruzi* surface glycoprotein GP72 in the insect and mammalian stages of the life cycle. *J. Cell Sci.* 106: 1023–1033.