16. R. L. Summitt, in *Trisomy 21 (Down's Syndrome)*, F. F. de la Cruz and P. S. Gerald, Eds. (University Park Press, Baltimore, 1981), p. 157.
17. G. Alimena *et al.*, *Cancer Genet. Cytogenet.* **16**, 207 (1985).
18. R. C. Strong, S. J. Korsmeyer, J. L. Parkin, D. C. Arthur, J. H. Kersey, *Blood* **65**, 21 (1985).
19. F. A. Salahuddin *et al.*, *Leukemia Res.* **6**, 729 (1982).
20. N. Sacchi, unpublished data.
21. C. Jones, D. Patterson, F. T. Kao, *Somatic Cell Genet.* **7**, 399 (1981).
22. J. N. Davidson *et al.*, in *Advances in Gene Technology: Human Genetic Disorders*, W. A. Scott *et al.*, Eds. (Cambridge Univ. Press, Boston, 1984), vol. 1, pp. 148–149.
23. H. A. Drabkin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 464 (1985).
24. H. A. Drabkin, unpublished data.
25. F. T. Kao and T. T. Puck, in *Methods in Cell Biology*, D. M. Prescott, Ed. (Academic Press, New York, 1984), vol. 8, pp. 23–29.
26. T. T. Puck and F. T. Kao, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 1227 (1967).
27. H. G. Morse, T. Hays, D. Patterson, A. Robinson, *Hum. Genet.* **61**, 141 (1982); B. Alhadeff, M. Velivasakis, M. Siniscalco, *Cytogenet. Cell Genet.* **19**, 236 (1977).
28. R. Watsons, M. Oskarsson, G. F. Vande Woude, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4078 (1982); P. A. Hieter, G. F. Hollis, S. J. Korsmeyer, T. A. Waldmann, P. Leder, *Nature (London)* **296**, 536 (1982).
29. M. LeBeau *et al.*, unpublished data.
30. M. O. Diaz, M. M. Le Beau, P. Pitha, J. D. Rowley, *Science* **231**, 265 (1986).
31. A. H. M. Geurts van Kessel, P. A. T. Tetteroo, A. E. G. Kr. von dem Borne, A. Hagemeijer, D. Bootsma, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3748 (1983).
32. P. Meera-Khan, *Arch. Biochem. Biophys.* **145**, 470 (1971); Van Someren *et al.*, *Humangenetik* **25**, 189 (1984).
33. J. Hoppener *et al.*, "Human gene mapping 8," *Cytogenet. Cell Genet.*, in press.
34. T. Maniatis, *et al.*, in *Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
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Binding of HTLV-III/LAV to T4$^+$ T Cells by a Complex of the 110K Viral Protein and the T4 Molecule

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Human T-lymphotropic virus type III (HTLV-III) or lymphadenopathy-associated virus (LAV) is tropic for human T cells with the helper-inducer phenotype, as defined by reactivity with monoclonal antibodies specific for the T4 molecule. Treatment of T4$^+$ T cells with monoclonal antibodies to T4 antigen blocks HTLV-III/LAV binding, syncytia formation, and infectivity. Thus, it has been inferred that the T4 molecule itself is a virus receptor. In the present studies, the surfaces of T4$^+$ T cells were labeled radioactively, and then the cells were exposed to virus. After the cells were lysed, HTLV-III/LAV antibodies were found to precipitate a surface protein with a molecular weight of 58,000 (58K). By blocking and absorption experiments, this 58K protein was identified as the T4 molecule. No cell-surface structures other than the T4 molecule were involved in the antibody-antigen complex formation. Two monoclonal antibodies, each reactive with a separate epitope of the T4 molecule, were tested for their binding capacities in the presence of HTLV-III/LAV. When HTLV-III/LAV was bound to T4$^+$ T cells, the virus blocked the binding of one of the monoclonal antibodies, T4A (OKT4A), but not of the other, T4 (OKT4). When HTLV-III/LAV was internally radiolabeled and bound to T4$^+$ T cells which were then lysed, a viral glycoprotein of 110K (gp110) coprecipitated with the T4 molecule. The binding of gp110 to the T4 molecule may thus be a major factor in HTLV-III/LAV tropism and may prove useful in developing therapeutic or preventive measures for the acquired immune deficiency syndrome.

Infection with the retrovirus referred to as human T-lymphotropic virus type III (HTLV-III) or lymphadenopathy-associated virus (LAV) results in a numerical and functional depletion of T helper/inducer cells (*1–3*). In its most severe form, infection is clinically manifest as susceptibility to opportunistic infections or malignancies and is known as the acquired immune deficiency syndrome (AIDS) (*1, 3–5*). The essential immunologic features of AIDS can be reproduced in cultures of normal human lymphocytes where HTLV-III/LAV infects, replicates in, and ultimately depletes T cells with the helper/inducer phenotype as defined by reactivity with monoclonal antibodies (T4, Leu 3, and CD4) (*1, 3–8*).

Apparently the T4 molecule on the cell surface of T helper/inducer cells is itself involved in the tropic interaction between T4$^+$ T cells and virus. Klatzman *et al.* (*7*) reported that viral replication was inhibited in vitro by incubating T cells with T4 monoclonal antibody (mAb), which indicates involvement of the T4 antigen in some part of the replication cycle. Dalgleish *et al.* (*8*) constructed a virus pseudotype between HTLV-III/LAV and vesicular stomatitis virus and demonstrated that treating target cells with CD4 mAb inhibited virus replication and syncytia formation (*8*). If the role of HTLV-III/LAV virus in the pseudotype virus is to permit virus penetration (*9*), these experiments provide more direct evidence that the T4 molecule is a virus receptor. In direct-binding studies, we have demonstrated that cell-surface binding of HTLV-III/LAV and T4A mAb reciprocally inhibit each other and that treating lymphocytes with T4A mAb before virus inoculation inhibits infection and virus replication (*10*). In the present studies, we demonstrate at a molecular level the coprecipitation of virus and the T4 molecule and show which virus structure or structures participate in the binding reaction.

In selecting a strategy for these experiments, we considered some results of binding studies (Table 1). If phytohemagglutinin (PHA)-stimulated normal human lymphocytes are treated with T4A mAb before being exposed to HTLV-III, binding of virus to these cells is inhibited. Treatment with T4 mAb, which binds to a separate site (epitope) of the T4 molecule (*11*), does not inhibit HTLV-III/LAV binding. Conversely, if the cells are first treated with HTLV-III/LAV, the binding of T4A mAb is inhibited but the binding of T4 mAb is not. Binding and binding inhibition results are the same whether performed on the T4$^+$ T-cell line CEM, on PHA-stimulated lymphocytes, or on unstimulated lymphocytes (*10*). Three prototype strains of virus (HTLV-III, LAV, and CDC-451) grown in PHA-stimulated lymphocytes or the H9 or CEM cell lines were tested, and all displayed T4 binding. [Reciprocal inhibition did not occur with any of a panel of other mAb's, including T3, T11, T8, Leu 8, T17, T10, Ia, Tac, 4F2, and T9, and inhibition with T4A mAb was not due to a reaction with the virus rather than with cells (*10*)]. Thus, with respect to a putative HTLV-III/LAV protein–T4 complex, the epitope recognized by T4 mAb on the complex should be accessible whereas the T4A epitope may not be.

A continuous human T4$^+$ T-cell line

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(CEM, subline E5) was surface-labeled with ^{125}I; the cells were incubated with virus, washed, and lysed with detergent buffer; and immunoprecipitations were performed on the lysates (Fig. 1a). Antibodies to HTLV-III/LAV coprecipitated a protein with a molecular weight of 58,000 (58K) (lane 1) which is the same molecular weight as the protein precipitated by T4A and T4 mAb's (lanes 7 and 8) (*12*). When HTLV-III/LAV binding was blocked by treatment of cells with T4A mAb, the 58K protein was not detected in HTLV-III/LAV–antibody precipitates (lane 4), whereas it was detected when the cells were treated with T4 mAb before virus incubation (lane 5). Furthermore, when the virus–T4 complex from lysates of virus-exposed cells was preabsorbed with T4A or T4 mAb linked to Sepharose, preabsorption with T4 mAb removed the 58K material that coprecipitates with virus and HTLV-III/LAV antibody whereas preabsorption with T4A mAb did not (Fig. 1b).

We then incubated radioactively labeled HTLV-III/LAV with CEM cells. The cells were washed and lysed, and immunoprecipitations were performed (Fig. 1c). Immunoprecipitation with T4 mAb coprecipitated a single protein with a molecular weight of 110,000 (gp110) (lane 1). Polyclonal antibody to HTLV-III/LAV precipitated the major proteins of the virus (lane 3) in a pattern that we believe is consistent with the label used ([^{35}S]methionine and [^{35}S]cysteine) and the source of the virus (extracellular as opposed to intracellular). By Western blot analysis of extracellular virus, the antibody to HTLV-III/LAV reacted with proteins of 13K, 17K, 24K, 31K, 39K, 41K, 51K, 66K, and 110K in titers ranging from 1:6400 to ≥ 1:1,000,000. Western blot or radioimmunoprecipitation analyses conducted with our serum in 11 other research laboratories produced similar results. The antiserum also precipitated a 160K protein when reacted with intracellular virus. The ^{35}S-labeled virus used in this study was harvested from extracellular culture supernatant and would be expected to have little or no 160K protein since this protein is the precursor for the 110K envelope protein and the 41K transmembrane protein (*13*). Similar results have been obtained by others who have compared intracellular with extracellular virus (*13*). We also did not detect the 51K protein. This protein is a precursor for *gag* region proteins (24K, 17K, and possibly 13K). Although the 51K protein is highly antigenic and often is detected by Western blot analysis, the actual protein content of the *gag* precursor in extracellular virus has been reported to be low or absent (*14*). The intensity of the 41K band is

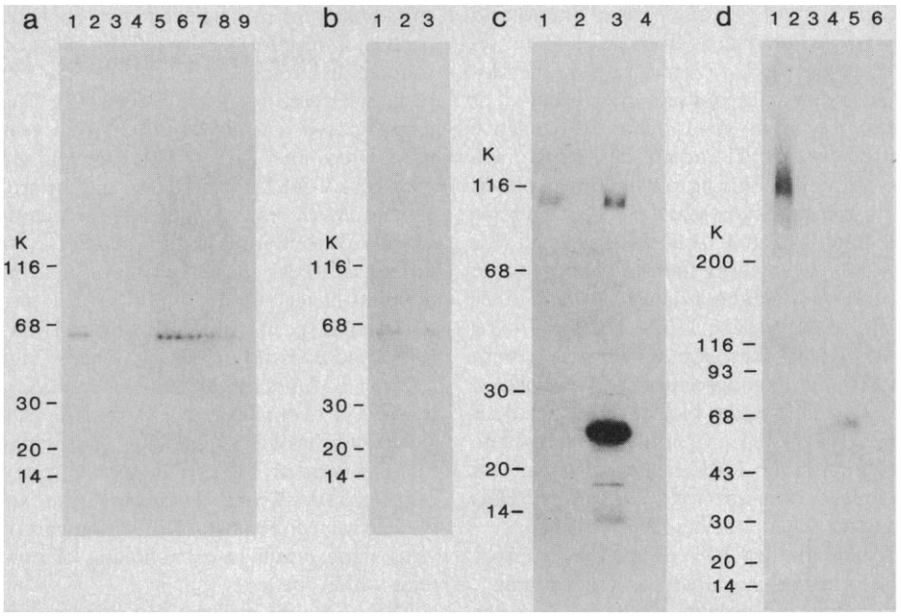

Fig. 1. (a and b) Coprecipitation of the T4 molecule with HTLV-III/LAV. Cells from the continuous human $T4^+$ T cell line, CEM (subline E5), were surface-labeled with ^{125}I by the lactoperoxidase technique (*24*). Labeled cells (10×10^7) were washed, split into nine tubes, and incubated with HTLV-III/LAV (4 μg in 40 μl) (*23*) for 30 minutes at 37°C. Subsequent washes and manipulations were performed at 0° to 4°C. Washed cells were lysed by adding 1.0 ml of detergent lysing buffer (LB) (*25*) and allowed to stand on ice for 15 minutes; the nuclei were removed by centrifugation at 3000*g* for 20 minutes. For absorptions, we prepared Sepharose conjugates of human HTLV-III/LAV and immune immunoglobulin G (IgG), nonimmune human IgG; the monoclonal antibodies T4A, T4, T11; and the murine paraprotein MOPC 141 (*26*). All lysates were absorbed with 200 μl of Sepharose–nonimmune human IgG for 1.5 hours with rotation, and then immunoprecipitated with 20 μl of Sepharose conjugates for 3 hours with rotation. The Sepharose absorbents were washed three times: once with LB; once with LB containing 0.5*M* NaCl; and once with LB containing 0.1 percent sodium dodecyl sulfate (SDS). Absorbed material was eluted at 65°C for 30 minutes with 20 μl of sample buffer [0.01*M* tris, *p*H 8.0, containing 2 percent SDS, 5 percent 2:mercaptoethanol (by volume), 25 μg of bromphenol blue per milliliter, and 10 percent glycerol (by volume)]. Electrophoresis was performed in a 3.3 percent to 20 percent gradient polyacrylamide gel with a 3 percent stacking gel (*27*), and autoradiographs were developed with Kodak XAR-5 film. (a) Lane 1, precipitate with the antibody to HTLV-III/LAV; lane 2, antibody to HTLV-III/LAV precipitated on lysed cells that had not been incubated with virus; lane 3, nonimmune human IgG precipitate. Immunoprecipitations with antibody to HTLV-III/LAV were also performed on lysates from virus-exposed cells that had been treated (before incubation with virus) with T4A mAb (lane 4), T4 mAb (lane 5), or T11 mAb (lane 6). In the remaining lanes, cell lysates (not incubated with virus) were precipitated with T4A mAb (lane 7), T4 mAb (lane 8), and T11 mAb (lane 9). T11 mAb and T4A mAb are the same Ig isotype (IgG2a). The T11 antigen is not expressed by CEM cells. (b) Lysates of virus-exposed cells were preabsorbed with Sepharose monoclonal protein absorbents before specific immunoprecipitation with antibody to HTLV-III/LAV: antibody to HTLV-III/LAV precipitated on lysates preabsorbed with T4A mAb (lane 1), T4 mAb (lane 2), or MOPC 141 (lane 3). T4 mAb and MOPC 141 are the same Ig isotype (IgG2b). (c) Coprecipitation of HTLV-III/LAV gp110 glycoprotein with the T4 molecule. The ^{35}S-labeled virus was prepared as follows: PHA-stimulated normal human lymphocytes that had been inoculated with HTLV-III/LAV 5 days previously (*17*) were washed, and 3×10^7 cells were suspended in 10 ml of cysteine and methionine-free RPMI medium containing 10 percent PHA (by volume) dialyzed fetal calf serum, [^{35}S]cysteine (100 μCi/ml), and [^{35}S]methionine (100 μCi/ml). The cells were incubated for 3 hours at 37°C, washed, and suspended in 30 ml of complete medium, and the culture supernatant was harvested 24 hours later. Virus was concentrated 100-fold by ultracentrifugation at 90,000*g* for 90 minutes over a cushion of 15 percent Renografin-60 (Squibb). ^{35}S-labeled virus (40 μl) was incubated with CEM cells (in 40 μl of medium, 7.5×10^6 cells per tube) for 30 minutes at 37°C. The cells were washed and lysed and treated as in (a) and (b). Lane 1, T4 mAb; lane 2, MOPC 141; lane 3, antibody to HTLV-III/LAV; lane 4, nonimmune human IgG. T4 mAb and MOPC 141 are the same Ig isotype (IgG2b). (d) Immunoprecipitation of the chemically cross-linked virus and cell surface protein complex with HTLV-III/LAV antibodies. PHA-stimulated normal human $T4^+$ T cells (5×10^7) (*10*) were radiolabeled and incubated with HTLV-III/LAV as described for (a) and (b). Virus-exposed cells were treated with dithiobis(succinimidyl) propionate (DSP), a chemical cross-linker that can be cleaved under reducing conditions (*16*): 5×10^6 cells per milliliter with DSP (50 μg/ml in PBS, *p*H 8.3, containing 1 m*M* $MgCl_2$, and 0.02 percent NaN_3) for 1 hour at 23°C. Washed cells were then lysed, and the lysates were preabsorbed, absorbed, eluted (with or without reducing agent, 2-mercaptoethanol, in the elution buffer), and electrophoresis was performed as for (a) and (b). Lanes 1 and 4, antibody to HTLV-III/LAV precipitated with lysates of virus-incubated cells (lane 1, nonreduced; lane 4, reduced). Lanes 2 and 5, control experiments with antibody to HTLV-III/LAV precipitated with lysates of cells that had not been incubated with virus (lane 2, nonreduced; lane 5, reduced). Lanes 3 and 6, control nonimmune IgG precipitates from lysates of virus-exposed cells (lane 3, nonreduced; lane 6, reduced).

relatively weak. On the basis of amino acid sequences predicted from nucleotide sequence data (*13, 15*), there are 26 methionine and cysteine residues in gp110 and, at most, seven in gp41. Thus, the relative intensities of gp41 and gp110 are not unexpected and are similar to what others obtain with the same radiolabels (*13*). The autoradiograph in Fig. 1c was overdeveloped (for 3 weeks) to intensify the weak bands in lane 3, and we still did not detect any band other than gp110 in lane 1. Finally, the T4 (or T4A) mAb does not react directly with gp110 in immunoprecipitation with labeled virus or in Western blot analysis, nor does the HTLV-III/LAV antibody precipitate anything in the 110K range from lysates of radiolabeled, uninfected CEM cells or PHA-stimulated lymphocytes.

Although the CEM cell line has the essential structures required for virus binding, infectivity, and replication, this line does not express all the cell surface structures of normal $T4^+$ T cells (for example, T11 and T3). It is also possible that, in addition to T4, another molecule is weakly associated with the T4–virus complex but is not recovered in the specific immunoprecipitates because of dissociation in the lysing buffer or during the manipulations. We treated virus-incubated cells with a bifunctional chemical cross-linker, dithiobis(succinimidyl) propionate (DSP). This compound covalently cross-links primary and secondary aliphatic amines, is reactive at physiologic *p*H, and contains an internal disulfide group that can be cleaved with reducing agents (*16*). The virus-exposed, chemically cross-linked cells were lysed, the lysates were precipitated with HTLV-III/LAV antibody, and the precipitates were resolved by SDS–polyacrylamide electrophoresis under reducing conditions (to break the internal S–S bond in the cross-linker). Only the 58K protein was observed in specific precipitates from PHA-stimulated normal human $T4^+$ T cells (Fig. 1d, lane 4). Under nonreducing conditions, the virus-T4 complex had an aggregate molecular weight of 320K to 400K (maximum intensity band at 360K). The stoichiometry of the gp110-T4 complex cannot be precisely determined because DSP treatment of virus alone results in cross-linking of proteins within the virus.

These results indicate that binding of HTLV-III/LAV to $T4^+$ T cells is due to a complex between gp110 and the T4 molecule. Results with two mAb's reactive with different epitopes of the T4 molecule indicate that binding occurs in only a portion of the T4 molecule. Whether the same is true for the gp110 protein is unknown, as is the location of the epitope.

Antibodies to the T4 molecule inhibit HTLV-III/LAV infectivity for $T4^+$ T cells (*8–10*), and sera from HTLV-III/LAV–infected subjects exhibit some capacity to inhibit (neutralize) HTLV-III/LAV infectivity in vitro (*17, 18*). Thus, immunologic intervention directed toward binding sites of the gp110–T4 complex may be a promising approach to immunotherapy or vaccine development. However, the inhibition of infectivity by T4A mAb or HTLV-III/LAV antibodies, in our hands, is not absolute. We obtain a 90 to 99.9 percent reduction in infectious virus titer in vitro (*10, 17*). This reduction may not be sufficient to limit an established infection in vivo since infection and disease often progress despite the presence of antibody, including antibody to gp110 (*19*), in HTLV-III/LAV–infected subjects. However, antibody may help limit the pace at which viral replication and $T4^+$ T cell depletion occur and, thus, determine the balance between stable and progressive disease. Even if antibody does not mediate resolution of established infection, it remains possible that antibody (induced by vaccination) would be effective in preventing infection from an initial exposure to infectious virus where the virus burden and inoculum may be substantially lower than in an established infection. Moreover, it is possible that the precise epitope on gp110 involved in the gp110-T4 complex is only marginally immunogenic when inoculated as intact virion and that maneuvers directed toward the precise epitope would be more effective than present observations suggest.

The extent of the genomic diversity between isolates of HTLV-III/LAV, much of which is in the envelope (*env*) region that codes for gp110 (*20*), is unknown. If (putative) protective immunity is strain-specific, construction of a vaccine will be more complicated. Furthermore, protective antibody could conceivably select for antibody-nonreactive pathogenic mutants (*21*). However, if all pathogenically important strains of HTLV-III/LAV display $T4^+$ T-cell tropism and if the gp110–T4 interaction is critical to that tropism, it seems to us that this region of gp110 is likely to be conserved and that antibody directed against the critical epitope (or epitopes) should be broadly reactive with HTLV-III/LAV isolates.

It is also possible that there are mechanisms of infection that are independent of the gp110–T4 interaction or that antibody reactive with the gp110 epitope, while preventing $T4^+$ T-cell infection, would divert or sequester virus (rather than destroy it) for replication or latency in other cell types. Nevertheless, it appears that $T4^+$ T-cell tropism is important in governing quantitative viral replication and pathogenesis, and that further studies of the gp110–T4 interaction should be conducted.

Table 1. Inhibition of binding of T4A (but not T4) by HTLV-III/LAV and of HTLV-III/LAV by T4A (but not T4). The binding of HTLV-III/LAV (tubes 1 to 5) or of mAb's (tubes 6 to 13) to PHA-stimulated normal human lymphocytes (5×10^5) (*17*) was determined by indirect immunofluorescence with a fluorescence-activated cell sorter (*10*). For inhibition of HTLV-III/LAV binding, cells were incubated (30 minutes) with mAb's (25 ng in 25 μl) (*22*) before the addition of HTLV-III/LAV (1 μg in 10 μl) (*23*). For inhibition of mAb binding, cells were incubated (30 minutes) with HTLV-III/LAV (1 μg in 10 μl) before the addition of mAb's (2.5 ng in 25 μl). The T11 mAb detects the E-rosette receptor on T cells and is the same immunoglobulin isotype (IgG2a) as T4A mAb. In binding studies performed with cells from numerous donors ($n > 20$), T4-enriched or T4-depleted cell populations, or continuous cell lines, the percentage of cells that bound virus and the percentage of cells that expressed T4 were similar (within 5 percent of each other).

Tube	First addition	Second addition	Number of cells fluorescence positive (%)
	Binding revealed by fluorescein-labeled antibody to HTLV-III/LAV		
1	Buffer	HTLV-III/LAV	46.2
2	T4A	HTLV-III/LAV	3.7
3	T4	HTLV-III/LAV	47.0
4	T11	HTLV-III/LAV	47.8
5	Buffer	HTLV-III/LAV	1.1
	Binding revealed by fluorescein-labeled antibody to mouse Ig		
6	Buffer	T4A	49.5
7	HTLV-III/LAV	T4A	0.7
8	Buffer	T4	46.7
9	HTLV-III/LAV	T4	48.8
10	Buffer	T11	85.5
11	HTLV-III/LAV	T11	83.9
12	Buffer	Buffer	0.4
13	HTLV-III/LAV	Buffer	0.6

REFERENCES AND NOTES

1. S. M. Broder and R. C. Gallo, *N. Engl. J. Med.* **311**, 1292 (1984).
2. J. J. Goedert *et al.*, *Lancet* **1984-II**, 711 (1984); J. Laurence *et al.*, *N. Engl. J. Med.* **311**, 1269 (1984); H. C. Lane *et al.*, *ibid.* **313**, 79 (1985); J. K. A. Nicholson *et al.*, *Ann. Intern. Med.* **103**, 37 (1985).
3. A. S. Fauci *et al.*, *Ann. Intern. Med.* **102**, 800 (1985).
4. M. Popovic *et al.*, *Science* **224**, 497 (1984).
5. F. Barré-Sinoussi *et al.*, *ibid.* **220**, 868 (1983); R. C. Gallo *et al.*, *ibid.* **224**, 500 (1984); J. Schüpbach *et al.*, *ibid.* p. 503; M. G. Sarngadharan, M. Popovic, L. Bruch, J. Schüpbach, R. C. Gallo, *ibid.* p. 506.
6. D. Klatzman *et al.*, *ibid.* **225**, 59 (1984); M. Popovic, E. Read-Connole, R. C. Gallo, *Lancet* **1984-II**, 1472 (1984).
7. D. Klatzman *et al.*, *Nature (London)* **312**, 767 (1985).
8. A. G. Dalgleish *et al.*, *ibid.*, p. 763.
9. J. Zavada, *J. Gen. Virol.* **15**, 183 (1972); P. Clapham, K. Nagy, R. A. Weiss, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2886 (1984).
10. J. S. McDougal *et al.*, *J. Immunol.* **135**, 3151 (1985).
11. T. C. Fuller *et al.*, *Human Immunol.* **9**, 89 (1984).
12. C. Terhorst, A. van Agthoven, E. Reinherz, S. Schlossman, *Science* **209**, 520 (1980).
13. J. S. Allan *et al.*, *ibid.* **228**, 1091 (1985); F. D. Veronese *et al.*, *ibid.* **229**, 1402 (1985).
14. J. Schüpbach *et al.*, *ibid.* **224**, 503 (1984).
15. L. Ratner *et al.*, *Nature (London)* **313**, 277 (1985).
16. M. B. Breuner, I. S. Trowbridge, J. L. Strominger, *Cell* **40**, 183 (1985).
17. J. S. McDougal *et al.*, *J. Immunol. Methods* **76**, 171 (1985).
18. R. A. Weiss *et al.*, *Nature (London)* **316**, 69 (1985); M. Robert-Guroff, M. Brown, R. C. Gallo, *ibid.* p. 72.
19. F. Barin *et al.*, *Science* **228**, 1094 (1985).
20. G. M. Shaw *et al.*, *ibid.* **226**, 1165 (1984); F. Wong-Staal *et al.*, *ibid.* **229**, 759 (1985).
21. O. Narayan, J. E. Clements, D. E. Griffin, J. S. Wolinsky, *Infect. Immun.* **32**, 1045 (1981).
22. Monoclonal antibodies and paraproteins used in this study were T4A, T4, T11 (OKT4A, OKT4, OKT11, Ortho Pharmaceutical) and the murine plasmacytoma paraprotein MOPC 141 (Litton Bionetics).
23. The HTLV-III/LAV preparation (LAV1 prototype strain) was derived from culture supernatants of HTLV-III/LAV–infected, PHA-stimulated lymphocytes (*17*), which were harvested by sequential centrifugation (300*g* for 7 minutes, followed by 1500*g* for 20 minutes) and concentrated 1000-fold by ultracentrifugation at 90,000*g* for 90 minutes over a cushion of 15 percent Renografin-60 (Squibb).
24. O. Acuto *et al.*, *Cell* **34**, 717 (1983). Precise conditions were 5×10^7 cells per milliliter in phosphate-buffered saline (PBS), *p*H 7.4, containing 1 mCi of $Na^{125}I$ per milliliter and 20 μg of lactoperoxidase per milliliter. At times 0, 1, 5, and 10 minutes, 10 μl of 0.03 percent H_2O_2 was added. Reaction was at 23°C and was stopped at 15 minutes by two centrifugations in 50 volumes of cold PBS containing 10 m*M* NaI.
25. Lysing buffer is 0.02*M* tris, 0.12*M* NaCl, *p*H 8.0, containing 0.2 m*M* phenylethylsulfonyl fluoride, aprotinin (5 μg/ml), 0.2 m*M* EGTA, 0.2 m*M* NaF, 0.2 percent sodium deoxycholate, and 0.5 percent Nonidet P-40 (by volume).
26. The human sera (nonimmune and HTLV-III/LAV–immune) were absorbed with CEM cells, and IgG fractions were prepared (*17*). Human IgG was coupled to Sepharose 4B (Pharmacia) by using CNBr [J. Porath, R. Axen, S. Ermback, *Nature (London)* **215**, 1491 (1967)]. These absorbents contained 4 to 5 mg of IgG per milliliter of Sepharose. Monoclonal antibody and the MOPC 141 absorbents are washed preparations of Sepharose CL–Protein A (2 mg of Protein A per milliliter of Sepharose; Pharmacia) that had been incubated with ≥5 mg of monoclonal protein per milliliter of Sepharose-Protein A.
27. D. M. Neville and H. Glossman, *Methods Enzymol.* **32**, 92 (1979).
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Prostacyclin Stimulation of the Activation of Blood Coagulation Factor X by Platelets

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When platelets were incubated with prostacyclin, prostaglandin E_1, or prostaglandin D_2 at concentrations insufficient to increase the level of adenosine 3′,5′-monophosphate (cyclic AMP), coagulation factor X was activated by a platelet cysteine protease. Prostacyclin or prostaglandin E_1 at higher concentrations increased the cyclic AMP level and inhibited the activation of factor X by platelets. Inhibition of platelet adenylate cyclase by 2′,5′-dideoxyadenosine allowed the activation of the protease at higher concentrations of the autocoids. Prostaglandins A_1, A_2, B_1, B_2, E_2, $F_{2\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$, and thromboxane B_2, which do not affect platelet cyclic AMP level, did not stimulate the protease.

THE BLOOD COAGULANT FACTOR Xa plays a pivotal role in the blood coagulation process through the formation of the prothrombinase complex (*1*). We showed earlier that not only is the activation of factor X a critical step in coagulation, but the activated factor is a potent inhibitor of thromboxane A_2 synthesis in platelets (*2*) and prostacyclin (PGI_2) synthesis in endothelial cells (*3*). During blood coagulation, factor Xa is produced by a limited proteolysis of factor X by two different but interrelated pathways known as the intrinsic and extrinsic pathways (*4*). Factor X is also reported to be activated by a Ca^{2+}-dependent activator present on the platelet surface (*5*).

Prostacyclin, prostaglandin E_1 (PGE_1), and PGD_2 are known for their ability to inhibit platelet aggregation by increasing the intracellular level of adenosine 3′,5′-monophosphate (cyclic AMP) but do not seem to have any effect on the activation of blood coagulation factors (*5, 6*). Now, we report that the exposure of human blood platelets to amounts of these prostaglandins that are insufficient to increase cyclic AMP levels results in rapid stimulation of a protease or proteases that activate factor X. Higher concentrations of these prostaglandins inhibit platelet-dependent activation of factor X as a result of the increase in cyclic AMP. These findings indicate the existence of a pathway by which prostaglandins induce the activation of factor X in platelets.

Addition of 2 n*M* PGI_2 or PGE_1 to a mixture of gel-filtered platelets and purified bovine factor X resulted in the generation of factor Xa in the presence of purified bovine factor Va, as indicated by hydrolysis of S-2222 (Fig. 1) or coagulation assay (*7*). Human factor X was similarly activated. No activity was observed if prostaglandin, platelets, or factor X was omitted. There was no effect on the activity of factor Xa (0.4 unit/ml) when either 2 n*M* or 10 n*M* PGI_2 or PGE_1 was added to the assay mixture. Other investigators have shown that the catalytic activity of factor Xa in the conversion of prothrombin to thrombin is enhanced by the interaction of protease with the platelet surface factor Va, which acts as the receptor of factor Xa (*8*). We found that the addition of factor Va increased by approximately tenfold the amidolytic activity of factor Xa, as compared with controls containing no factor Va. Factor Va itself has no effect either on the hydrolysis of S-2222 or on the activation of factor X. The addition of factor Xa inhibitors like soybean trypsin inhibitor (23 μ*M*) or phenylmethylsulfonyl fluoride (2 m*M*) to the reaction mixture inhibited the hydrolysis of S-2222 by 100 and 95 percent, respectively, indicating the generation of factor Xa in the assay mixture.

In a separate experiment, diisopropyl phosphofluoridate derivative of factor X was prepared as described previously except that factor Xa was replaced by the zymogen (*2*). When factor X was replaced by the inhibitor-treated zymogen in the incubation mixture, no factor Xa activity was seen, as compared with the controls. Since diisopropyl phosphofluoride is a covalent inhibitor of factor Xa (*1*), the prostaglandin-mediated

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