bath solution. High K^+ solutions were prepared by substituting KCl for NaCl.

9. Neuronal $[Ca^{2+}]_i$ was analyzed with fura 2–acetoxymethyl ester (AM) as described [G. Grynkiewicz *et al.*, *J. Biol. Chem.* **260**, 3440 (1985); D. A. Williams *et al.*, *Nature* **318**, 558 (1985); J. A. Connor *et al.*, *J. Neurosci.* 7, 1384 (1987); J. A. Connor *et al.*, *Science* **240**, 649 (1988); C. S. Cohan, J. A. Connor, S. B. Kater, *J. Neurosci.* 7, 3588 (1987); M. P. Mattson, M. Murrain, P. B. Guthrie, S. B. Kater, *ibid.* **9**, 3728 (1989)]. After adding Eagle's minimum essential medium containing 10 μ*M* fura 2–AM to retinal or hippocampal cell neurons, the cultures were incubated at 37°C in a 5% CO_2/95% air humidified chamber and then rinsed. The dye was loaded, trapped, and deesterified within 1 hour, as determined by stable fluorescence ratios and the effect of the Ca^{2+} ionophore ionomycin on measured $[Ca^{2+}]$. During Ca^{2+} imaging, the cells were incubated in a solution of Hepes-buffered saline with Hanks balanced salts (*8*). The $[Ca^{2+}]_i$ was calculated from ratio images that were obtained by measuring the fluorescence at 500 nm that was excited by 350 and 380 nm with a DAGE MTI 66 SIT camera mounted on a Zeiss Axiovert 35 microscope. Exposure time for each picture was 500 ms. Analysis was performed with a Quantex (Sunnyvale, CA) QX7-210 image-processing system. Since cells were exposed to ultraviolet light only during data collection (generally less than a total of 20 s per cell), bleaching of fura 2 was minimal.
10. Recombinant gp120 was produced by transfection of a Chinese hamster ovary (CHO) cell line with a plasmid containing the 3B envelope coding sequences from amino acids 61 to 531 [L. A. Lasky *et al.*, *Science* **233**, 209 (1986)]. The gene was truncated in this fashion to remove the native NH_2-terminal signal sequence and the COOH-terminal hydrophobic domains. This fragment was then ligated in-frame to the herpes simplex virus glycoprotein D signal sequence [P. W. Berman, T. Gregory, D. Crase, L. A. Lasky, *Science* **227**, 1490 (1985)] to allow the envelope protein to be constitutively secreted by the CHO cell line. Production in a mammalian cell ensured that the envelope protein was glycosylated. This envelope glycoprotein, rgp120-3B, was purified by immunoaffinity chromatography to greater than 99.9% purity.
11. E. B. Dreyer, P. K. Kaiser, S. A. Lipton, unpublished observations. Native gp120 was purified by immunoaffinity chromatography from two natural isolates, RF2 and 3B (*7, 13*) [S. W. Pyle *et al.*, *AIDS Res. Hum. Retrovir.* **3**, 387 (1988)]. The concentration of gp120 in the purified material was approximately 50% based on estimates from polyacrylamide gel electrophoresis (PAGE) and immunoblotting.
12. Hippocampal cortices of embryonic day 18 CD rats were dissociated with trypsin (0.027% w/v) and plated at a density of 600,000 cells per 35-mm culture dish, each dish containing five poly-L-lysine–coated glass cover slips. Growth medium [P. A. Rosenberg and E. Aizenman, *Neurosci. Lett.* **103**, 162 (1989)] was changed three times per week. In these experiments, Ca^{2+} measurements were made after 14 to 21 days in culture.
13. Immunoprecipitation of gp120 was performed as described (*7*) with some modifications. A 1:100 or 1:500 dilution of anti-gp120 or preimmune serum from the same goat, was bound to protein-A–coated Sepharose beads, washed, and incubated with a solution containing 7 n*M* gp120 for 18 hours at 4°C; this was followed by centrifugation. The supernatant of the material treated with preimmune serum had gp120 activity as evidenced by immunoblotting (Fig. 2, inset) and by producing an increase in $[Ca^{2+}]_i$ and cell death (after a dilution of 1:350 to ~20 p*M*); the material exposed to anti-gp120 had little or no activity.
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15. After a 10- to 40-min incubation in low Ca^{2+} solution, 50 m*M* K^+ applied in the presence of Ca^{2+} still produced a significant increase in $[Ca^{2+}]_i$, although not of the same magnitude as that observed when neurons were not incubated in nominally Ca^{2+}-free medium (*16*). The effect of K^+ under these conditions shows that the neurons are still capable of responding to external stimuli that lead to an increased influx of Ca^{2+}.
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21. Before Ca^{2+} imaging and the application of gp120, retinal neurons were incubated for 1 hour at 37°C with ascites containing W3/25 (~2.5 μg/ml) and MRC OX-35 (~2.5 μg/ml), two specific antibodies directed against the rat CD4 molecule. These concentrations of the antibodies are known to block effects mediated by high-affinity binding to the CD4 protein on the surface of rat T cells [W. A. Jeffries, J. R. Green, A. F. Williams, *J. Exp. Med.* **162**, 117 (1985)]. These antibodies do not compete with one another for binding to CD4, so they could be used simultaneously.
22. S. A. Lipton, P. K. Kaiser, J. T. Offermann, N. J. Sucher, E. B. Dreyer, in preparation. Both native and recombinant viral coat protein gp120 from HIV-1 were found to be toxic in a dose-dependent fashion at picomolar concentrations to rat retinal ganglion cell neurons in culture. Control experiments, similar to those performed in the Ca^{2+} imaging experiments, suggested that the lethal effects of the purified preparations of the envelope protein were caused by gp120 and not by a contaminant: (i) neurotoxicity could be abated by anti-gp120, but not by control preimmune sera; (ii) trypsin neutralized the deleterious effect of gp120 on retinal ganglion cells; (iii) at similar doses, glycoprotein D did not injure the neurons; and (iv) antibodies against CD4 (anti-CD4) did not prevent gp120-induced neuronal cell injury in our culture system. Although picomolar concentrations of gp120 appear to have potent effects, the actual level of gp120 shed by the virus in AIDS patients is unknown.
23. A. Scriabine, personal communication. In the presence of 5% rat serum in the culture medium used in our toxicity experiments (*6*), ≥96% of the nimodipine was protein bound, yielding a free concentration in the low nanomolar range.
24. Dihydropyridine antagonists have been shown to be more effective on depolarized neurons [B. P. Bean, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6388 (1984); M. C. Sanguinetti and R. S. Kass, *Circ. Res.* **55**, 336 (1984); A. M. Brown, D. L. Kunze, A. Yatani, *J. Physiol. (London)* **379**, 495 (1986)]. Retinal ganglion cells lying among small clusters of other neurons are known to experience frequent depolarizations because of synaptic inputs [S. A. Lipton, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9774 (1986)]. For this reason, the clustered neurons were examined for effects of nimodipine in these experiments. In the absence of nimodipine, the levels of intracellular Ca^{2+} of clustered retinal ganglion cells before and after application of gp120 were slightly greater than the corresponding levels in solitary cells; however this difference was not statistically significant, and in any case had no bearing on these results.
25. S. A. Lipton *et al.*, unpublished data.
26. We thank H.-S. Vincent Chen and L. Dawes for helpful discussions, S. Pyle and L. Arthur of the National Cancer Institute for providing purified isolates of native gp120-RF2 and gp120-3B, and L. Lasky, T. Gregory, P. Berman, and L. Riddle of Genentech for recombinant gp120-3B. We also thank W. Cruikshank and H. Kornfeld of Boston University Medical School for instructing us in their immunoprecipitation technique, T.-H. Lee for anti-gp120 and preimmune serum generated in his laboratory at Harvard Medical School, and A. F. Williams at the University of Oxford for antibodies to rat CD4. We are grateful to P. A. Rosenberg for the use of his hippocampal cultures and to E. Aizenman for reading an earlier version of the manuscript. Supported in part by grants from the NIH (EY 05477 to S.A.L. and NS 01395 to E.B.D.), by the Burroughs Wellcome Fund and National Society to Prevent Blindness (to E.B.D.), by Fight-for-Sight, Inc. of New York City (to P.K.K.), and by an Established Investigatorship of the American Heart Association (to S.A.L.).

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Defective Presentation of Endogenous Antigen by a Cell Line Expressing Class I Molecules

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Cytotoxic T lymphocytes (CTLs) recognize class I major histocompatibility complex (MHC) molecules associated with antigenic peptides derived from endogenously synthesized proteins. Binding to such peptides is a requirement for class I assembly in the endoplasmic reticulum (ER). A mutant human cell line, T2, assembles and transports to its surface some, but not all, class I MHC molecules. The class I molecules expressed on the surface of T2 do not present peptides derived from cytosolic antigens, although they can present exogenously added peptides to CTL. The transported class I molecules may interact weakly with an unknown retaining factor in the ER such that they can assemble despite the relative shortage of peptides.

THE PEPTIDES THAT ASSOCIATE WITH class I MHC molecules derive from viral or other proteins synthesized within the antigen-presenting cell (APC) (*1*), although the final subcellular location of the protein does not determine whether the peptides are presented in this way (*2*). Class I MHC–associated peptides can also be derived from proteins experimentally introduced into the APC cytoplasm (*3, 4*). The peptide–class I complex can be mimicked by the exogenous addition of short, synthetic peptides to APCs, probably by direct binding to MHC molecules at the surface (*5*).

Antigenic peptides probably associate with class I molecules in the ER (*6*). A mutant murine cell line, RMA-S, which fails

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Table 1. FACS analysis of class I MHC expression on target cells. Cells grown in culture were washed once with Hanks balanced salt solution (HBSS) and then incubated with 100 μl of HBSS containing Y3 antibody to H-2K^b (anti–H-2K^b), BB7.2 (anti–HLA-A2), 4D12 (anti–HLA-B5), or no first antibody for 30 min at 4°C, washed once with HBSS, incubated with 100 μl of fluoresceinated antibody to mouse immunoglobulin Fc (FITC anti-Fc) in HBSS for 30 min at 4°C, washed once with HBSS, and fixed in 1% formaldehyde in phosphate-buffered saline. Single-color FACS analysis was performed on a FACS-4 (Becton Dickinson) and numbers represent the mean linear fluorescence of 10,000 events.

Cell line	Mean fluorescence intensity with antibody			
	FITC anti-Fc	Y3 (K^b)	BB7.2 (A2)	4D12 (B5)
T2	16.3	14.0	147.4	30.8
T2K^b	20.4	151.0	198.8	19.7
EL4-A2	5.7	201.7	62.1	10.5
Jurkat-A2	6.6	6.9	456.5	9.4
JY	14.2	10.2	650.6	19.2
Jurkat-K^b	6.0	157.2	8.0	8.8

Table 2. T2 is unable to present endogenously synthesized influenza matrix (M_1) protein to HLA-A2–restricted CTLs. Target cells (10^6) were washed twice with RPMI 1640 and resuspended in 5 ml of RPMI 1640. We added 200 μl of X31 [1000 hemagglutination units (HAU) per milliliter] influenza virus to target cells, and the cells were incubated for 2 hours at 37°C. Sodium [^{51}Cr]chromate (200 μCi) was added, and the cells were incubated at 37°C for an additional hour. The cells were then washed twice with RP10 (RPMI 1640 supplemented with 10% fetal bovine serum), resuspended at 10^5 cells per milliliter in RP10, and used in a ^{51}Cr-release assay. The effector CTL clone 219 specific for M_1 protein was derived from B10.D2 mice transgenic for a chimeric gene encoding the HLA-A2 $\alpha_1\alpha_2$ and the H-2K^b α_3 domains. The mice were immunized intraperitoneally with 300 HAU A/PR/8/34 influenza virus. Three weeks later, 5×10^6 primed spleen cells were restimulated in vitro once with 5×10^6 A/PR/8/34 infected, irradiated (3000 rads), HLA-A2/K^b transgenic spleen cells per well of a 24-well plate (Costar) in RP10 medium. Subsequent weekly restimulations were carried out with 1×10^5 to 2×10^5 irradiated (20,000 rads) Jurkat-A2 cells coated with 3 μg of M_{155-73} peptide per 10^6 cells and 5×10^6 irradiated (3000 rads) B10.D2 spleen cells per well of a 24-well plate in RP10 medium supplemented with 5% rat concanavalin A supernatant and 5% α-methyl mannoside (*16*). Effector cells (E) were incubated with target cells (T) (10^4) with or without 24 μ*M* M_{155-73} peptide at ratios of 4:1 or 0.4:1 in a ^{51}Cr-release assay for 6 hours at 37°C. Similar results were obtained in two other experiments.

Target cell	X31 virus infection	M_{155-73} peptide	Percent specific lysis by CTL 219 at E:T ratios of	
			4:1	0.4:1
EL4-A2	−	−	0	0
	−	+	84	42
	+	−	72	55
Jurkat-A2	−	−	10	17
	−	+	72	34
	+	−	47	28
JY	−	−	11	9
	−	+	37	15
	+	−	46	33
T2	−	−	0	0
	−	+	87	61
	+	−	0	0

to assemble class I molecules in the ER, can be induced to assemble, transport, and express its class I molecules at the surface by being cultured in a high concentration of peptides that bind the class I molecules (*7*). The T2 cell line was derived by fusion from the human mutant B lymphoblastoid line, LCL 721.174, which has a deletion in the class II region of the MHC (*8*). T2 transcribes and translates the genes for histocompatability antigens HLA-A2, HLA-B5, and β_2M. Only HLA-A2 is assembled and expressed at the cell surface. Other human class I genes (HLA-A3, Bw58, B7, and B8), after transfection into T2, behave like HLA-B5, and the products remain in the ER (*9*). Interestingly, three murine class I molecules (H-2K^b, D^p, and L^d) are assembled and transported to the surface after gene transfection into T2 (*9*). As a step toward understanding the distinction made between retained and transported class I molecules, we asked whether the HLA-A2 and H-2K^b molecules, which are expressed at the surface of T2, can present to CTL antigenic peptides derived from cytosolic proteins.

We compared T2 to normal human and mouse APCs transfected with H-2K^b or HLA-A2 to control for any nonspecific effects caused by class I expression in xenogeneic APCs. Control APCs included murine EL4 and human Jurkat tumor cells transfected with HLA-A2 (EL4-A2 and Jurkat-A2), Jurkat cells transfected with H-2K^b (Jurkat-K^b), and the HLA-A2 expressing human tumor cell line JY (*10*). All APCs expressed class I molecules on the surface at densities within a factor of 4 or 5 of the density on T2 (Table 1). Transfected APCs, including T2K^b, were analyzed by fluorescence-activated cell sorting (FACS) several times during the course of these experiments and maintained similar levels of class I expression throughout.

The first antigen for CTL that we investigated was the influenza matrix protein, M_1, which has been shown to be restricted by HLA-A2 (*11*). When either human (Jurkat-A2 or JY) or murine (EL4-A2) cell lines expressing HLA-A2 were infected with influenza X31 virus they became good targets for lysis by the M_1-specific CTL clone 219 (Table 2). However, T2 did not become a target for specific lysis by CTL 219 when infected with the X31 virus. This was not because T2 was not infected, because all APCs appeared to be infected by X31 when analyzed by SDS-PAGE (*12*). This was also not the result of a defect in HLA-A2 molecules made by T2, since T2 became a good target for CTL when incubated with exogenous M_{155-73} peptide (Table 2).

To rule out that the inability of T2 to present antigen was peculiar to an HLA-A2–restricted antigen, we investigated the ability of T2 to present two H-2K^b–restricted antigens, vesicular stomatitis virus nucleoprotein (VSV-N) and ovalbumin (OVA). VSV-N is the major cross-reactive antigen between VSV serotypes for specific CTLs in H-2^b mice and is H-2K^b–restricted (*13*). As expected, when either murine (EL4) or human (Jurkat-K^b) control APCs were infected with VSV they became good targets for both a VSV-N–specific CTL line, αVSV, and a CTL clone, 33 (Table 3). Both the αVSV CTL line and clone 33 also recognize the N1 cell line, EL4 cells transfected with the VSV-N gene (*13*). The T2K^b cell line infected with VSV, however, did not become a target for specific CTLs. Again this is not because VSV did not infect T2K^b, because all APCs tested appeared to be infected by VSV when analyzed by SDS-PAGE (*12*). It is also not as a result of H-2K^b expression by xenogeneic cells, because Jurkat-K^b APCs become good targets for CTLs when infected with VSV (Table 3).

Since the VSV-N antigenic peptide presented in association with H-2K^b has not yet been identified, it was not possible to test whether the H-2K^b molecules made by T2K^b were functional. Therefore, we tested the ability of T2K^b to present a second H-2K^b–restricted antigen, OVA, for which an antigenic peptide has been identified. When OVA is introduced into the APC cytosol by the osmotic lysis of pinosomes, it can be processed and presented to specific CTLs in the context of H-2K^b (*4*). APCs can also be sensitized for lysis by exogenous synthetic OVA peptides spanning residues 258 to

Table 3. T2K^b is unable to present endogenously synthesized VSV-N to H-2K^b–restricted CTLs. Target cells (10^6) were washed once in HBSS and resuspended in 2 ml of RPMI 1640 supplemented with 2% fetal bovine serum. We added 10 μl of VSV [5×10^9 plaque-forming units (PFU) per milliliter] to target cells. After incubation for 1 hour at 37°C, 200 μCi of sodium [^{51}Cr]chromate was added, and the cells were incubated for an additional hour at 37°C. Target cells were then washed twice with RP10 and used in a ^{51}Cr-release assay. The anti-VSV CTL line was derived from C57BL/6 mice primed intraperitoneally with 10^6 PFU VSV virus. The primed spleen cells (30×10^6) were restimulated in vitro with 30×10^6 VSV infected (multiplicity of infection of 1), irradiated (3000 rads) C57BL/6 spleen cells in RP10 in upright 25-cm^3 flasks. Thereafter 5×10^5 anti-VSV CTLs were restimulated with 10^5 irradiated (20,000 rads) N1 cells and 5×10^6 irradiated (3000 rads) C57BL/6 spleen cells per well of a 24-well plate. The VSV-N–specific CTL clone 33 was derived from C57BL/6 mice and maintained as described (*17*). Effector cells were incubated with target cells (10^4) at ratios of 7.6:1 and 0.8:1 for anti-VSV CTLs and 6:1 and 0.7:1 for CTL clone 33 in a ^{51}Cr-release assay for 3 hours at 37°C. Similar results were obtained in six other experiments.

Target cell	VSV	Percent specific lysis by CTLs			
		Anti-VSV		33	
		7.6:1	0.8:1	6:1	0.7:1
EL4	−	0	2	4	4
	+	92	67	84	40
Jurkat-K^b	−	3	0	0	0
	+	90	49	79	54
T2K^b	−	3	0	0	0
	+	1	0	0	0
N1*	−	85	56	80	57

*N1 is the EL4 cell line transfected with the VSV-N gene (*13*).

Table 4. T2K^b is unable to present OVA introduced into the cytosol to H-2K^b–restricted CTLs. Target cells (10^6) to be loaded with OVA were pelleted and resuspended in 200 μl of warm hypertonic medium [0.5*M* sucrose, 10% (w/v) polyethylene glycol 1000, and 10 m*M* Hepes in RPMI 1640 (*p*H 7.2)] containing OVA (3 mg/ml). After incubation for 10 min at 37°C, 15 ml of warm hypotonic medium [60:40 (v/v) RPMI 1640:H_2O] was added, and the cells were incubated at 37°C for an additional 2 min (*4*). All target cells were then pelleted and labeled with 100 μCi sodium [^{51}Cr]chromate in 1 ml RP10 for 1 hour at 37°C. The cells were then washed twice with HBSS resuspended at 10^5 cells per milliliter in RP10, and used in a ^{51}Cr-release assay. The OVA-specific CTL clone B3 was derived from C57B1/6 mice primed with the $OVA_{229-276}$ peptide as described (*18*). Effector cells (E) were incubated with target cells (T) (10^4) with or without 0.01 m*M* $OVA_{229-276}$ peptide in a ^{51}Cr-release assay for 3 hours at 37°C at ratios of 10:1 and 1:1. Similar results were obtained in seven other experiments.

Target cell	OVA load	$OVA_{229-276}$ peptide	Percent specific lysis by CTL B3 at E:T ratios	
			10:1	1:1
EL4	−	−	0	0
	−	+	78	35
	+	−	84	44
Jurkat-K^b	−	−	1	0
	−	+	86	63
	+	−	45	34
T2K^b	−	−	1	3
	−	+	68	49
	+	−	7	1
E.G7-OVA*	−	−	95	46

*E.G7-OVA is the EL4 cell line transfected with the OVA cDNA (*4*).

276. In agreement with these observations, we found that when either murine (EL4) or human (Jurkat-K^b) APCs were loaded with OVA, they became good targets for the OVA-specific CTL clone B3 (Table 4). However, when T2K^b cells were loaded with OVA they did not become targets for lysis. This is not because the H-2K^b molecule expressed by T2 cannot present antigen to specific CTLs, since both T2K^b and control murine and human APCs become sensitized for lysis by exogenous synthetic OVA peptide. The difference in the ability of T2K^b to become sensitized for lysis does not appear to be simply quantitative, since loading of T2K^b with larger amounts of OVA gives the same results (*12*).

We have shown that HLA-A2 and H-2K^b molecules assemble and traffic to the cell surface in T2 cells even though they do not associate with peptides derived from the three cytoplasmic antigens we investigated (influenza matrix, VSV-N, and OVA). It is likely then that T2 does not provide these peptides to the ER, which results in the retention of most human class I molecules. Candidates for proteins whose loss might lead to peptide deficiency in the ER include the heat-shock protein family, which has been shown to bind peptides in an adenosine triphosphate (ATP)–dependent manner and might function to bind and transport antigenic peptides (*14*). Another candidate is a protein specialized for transport of peptides into the ER (*7*).

In addition, there is a factor in T2 that results in the differential transport of mouse and human class I molecules, with the exception of HLA-A2. Chimeric class I genes have mapped the distinction made by T2 between mouse and human class I molecules to the α_1 and α_2 domains of the heavy chain (*15*). Coincidentally, these domains also form the peptide-binding site on the class I molecule and appear to require peptides to assemble in the RMA-S cell line (*7*). The mechanism for this factor that is most consistent with these observations is that of a chaperonin-like protein (CHAP) in T2 with a function dependent on interaction with the $\alpha_1\alpha_2$ domains of class I molecules in the ER (*15*). The interaction of CHAP with class I molecules is normally disrupted by the binding of antigenic peptides to their restricting MHC molecules in the ER, resulting in the transport of native, peptide-containing class I molecules to the cell surface. In the mutant mouse cell line RMA-S, mouse class I molecules are retained by mouse CHAP because antigenic peptides are deficient in the ER. In the human mutant cell line T2, most human class I molecules are retained in a similar manner. Human CHAP would probably have a lower affinity for mouse class I molecules, because the two molecules have not evolved together. Thus, mouse class I molecules would not be retained in the ER of T2 even in the absence of antigenic peptides from the cytosol. Thus mouse class I heavy chains can assemble with β_2M in the ER of T2 and be transported to the cell surface.

If T2 has a deficiency of cytosolic-derived antigenic peptides in the ER and transports some, but not all, class I molecules to the cell surface, one might ask whether the class I molecules that are transported are devoid of peptides. By comparing the ability of formalin fixed and untreated APCs to present different concentrations of peptides to CTLs, however, we find no evidence that the peptide binding sites of the class I molecules expressed by T2 are empty. These results are consistent with the idea that class I molecules require peptides for assembly and transport to occur, and we cannot rule out that such peptides might arise from degradation of proteins within the secretory pathway or from peptides available in the medium. To date only cytosolic antigens have been tested for presentation by T2 to specific CTLs. It will be of interest to see whether antigens localized to the ER can overcome the inability of T2 to present endogenous antigen simply by virtue of their location.

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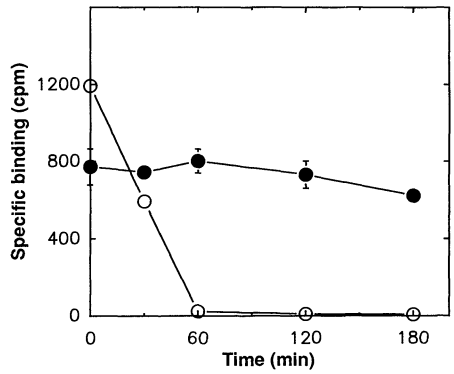

Fig. 1. Inhibition of ^{125}I–TNF-α, but not ^{125}I–IFN-γ, binding to macrophages after incubation with taxol. Thioglycollate-elicited peritoneal macrophages (CD1, 8 weeks, Charles River) were incubated with taxol (10 μ*M*) at 37°C for indicated times. The cells were washed, and ^{125}I–TNF-α (○) or ^{125}I–IFN-γ (●) binding was then measured at 4° or 25°C, respectively, for 3 hours as described (*5, 15*). Results are means ± SE from one of three similar experiments.

Shared Actions of Endotoxin and Taxol on TNF Receptors and TNF Release

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Bacterial lipopolysaccharide (LPS) exerts profound effects on mammalian hosts in part by inducing macrophages to release tumor necrosis factor–α (TNF-α); the mechanisms involved are unresolved. The microtubule stabilizer taxol shared two actions of LPS on macrophages: it rapidly decreased TNF-α receptors and triggered TNF-α release. Both actions of taxol were absent in LPS-hyporesponsive C3H/HeJ mice. In recombinant inbred mice, the genes controlling responses to LPS and to taxol were closely linked. Dexamethasone blocked release of TNF-α by both stimuli but did not block the decrease in TNF-α receptors. Thus, a protein associated with microtubules may be a cellular target of LPS.

ENDOTOXIC LIPOPOLYSACCHARIDE, the major component of the outer membrane of Gram-negative bacteria, has profound effects on eukaryotic cells and mammalian organisms (*1*). Among its primary targets are macrophages, which respond to LPS by releasing inflammatory mediators, such as TNF-α, interleukin-1, interleukin-6, interferon-α, interferon-β, platelet activating factor, eicosanoids, and reactive oxygen and nitrogen intermediates (*2*). TNF-α is an endogenous mediator of septic shock (*3*). However, it is not clear to what intracellular targets LPS binds (*4*), or how LPS initiates TNF-α production or any other cellular effect. Macrophages respond to TNF-α by means of specific receptors (TNF-R) (*5*). The LPS decreases macrophage TNF-R expression so rapidly that secretion of TNF-α and its binding to TNF-R could account for only a small part of the effect (*5*). We recently observed that microtubule-depolymerizing agents also decrease TNF-R by a mechanism not involving TNF-α release (*6*). Taxol is a plant-derived antitumor drug (*7*) that binds polymerized tubulin stoichiometrically and reversibly at a site different from colchicine, podophyllotoxin, vinblastine, or guanine nucleotides and thereby inhibits depolymerization (*8*). We now show that taxol acts on macrophages to mimic two effects of LPS: to decrease TNF-R and to induce TNF-α release.

Taxol (10 μ*M*, 37°C) inhibited the binding of ^{125}I-labeled TNF-α (^{125}I–TNF-α) to macrophages by 50% within 30 min, as measured at 4°C. By 1 hour, we could detect no TNF-α binding sites (Fig. 1). Binding of interferon-γ (IFN-γ) was unaffected by taxol (Fig. 1), similar to the insensitivity of the IFN-γ receptor to LPS (*9*). A 1-hour incubation with taxol at 4°C before, or addition of taxol during, the binding step did not diminish binding of ^{125}I–TNF-α (*10*). By a limulus test, 10 μ*M* taxol contained too small an amount of LPS (<1 pg/ml) to decrease TNF-R (*5*).

To see if taxol was inducing TNF-α release, we used an antiserum (15E) against murine TNF-α (*11*). When present during incubation with taxol, 15E partially reversed the inhibitory effect of taxol on ^{125}I–TNF-α binding (Fig. 2A). This suggested that taxol triggered TNF-α release, but that binding of endogenous TNF-α was only partially responsible for decreased binding of ^{125}I–TNF-α. Induction of TNF-α by taxol was confirmed by a radioreceptor assay (*12*). Macrophage-conditioned media from the culture assayed in Fig. 1 were collected and tested for their ability to compete with ^{125}I–TNF-α for binding sites at 4°C, a condition in which the indicator cells could not respond to taxol. These conditioned media blocked binding of ^{125}I–TNF-α (Fig. 2B). Independent evidence was provided by using flow cytometry to measure the fluorescence of saponin-permeabilized murine macrophages stained with a monoclonal antibody to TNF. Staining for intracellular TNF-α was induced by both taxol and LPS (Fig. 2C).

To test if responses to LPS and taxol share a genetic basis, we examined LPS-hyporesponsive C3H/HeJ mice, whose gene defect has been mapped to the fourth chromosome (LPS^d) (*13*). TNF-R expression on C3H/HeJ macrophages was unaffected by taxol (10 μ*M*), whereas on macrophages from the closely related LPS-responsive strain C3H/HeN (LPS^n) TNF-R surface expression was decreased by 50% with ~0.15 μ*M* taxol and by >95% with 1 μ*M* taxol (Fig. 3). In contrast, exogenous TNF-α decreased

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