evoked by 100 μM Glu, suggesting that all three are full agonists.
16. The oocytes were treated with PTX (4 μg/ml) in Barth's medium [88 mM NaCl, 1 mM KCl, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 2.4 mM $NaHCO_3$, 10 mM Hepes, pH 7.4] for 24 hours at 19°C [N. Dascal *et al.*, *Mol. Brain Res.* **1**, 201 (1986); T. M. Moriarty *et al.*, *J. Biol. Chem.* **264**, 13524 (1989)].
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13 February 1991; accepted 5 April 1991

Technical Comments

CD14 and Immune Response to Lipopolysaccharide

Lipopolysaccharide binding protein (LBP) is a normal serum component that can bind to soluble lipopolysaccharide (LPS) and to LPS that is expressed on bacterial surfaces (*1*). Binding of the LPS-LBP complex to macrophages induces production of tumor necrosis factor (TNF)(*2*), which is a primary mediator of endotoxic shock. Moreover, LBP concentration increases after induction of the acute phase response (*1*). These results suggest that LBP has a major regulatory role in Gram-negative bacterial clearance and in the destructive processes of LPS-induced schock. Wright *et al.* reported (*3*) that CD14, a 55-kD glycoprotein expressed by monocytes and macrophages, is a cell surface receptor for LBP-LPS. Induction of TNF release by LBP-LPS complexes was blocked by anti-CD14 monoclonal antibodies in a highly specific manner. Thus, CD14 expression represents a second mechanism by which the immune response to LPS might be regulated.

In 1985, Maliszewski *et al.* reported (*4*) on the purification and biochemical characterization of My23, a 55-kD protein on monocyte cell surfaces that was recognized by the monoclonal antibody AML-2-23. (The My23 antigen was later designated "CD14" by the International Workshops on Leukocyte Antigens.) We demonstrated that a soluble form of CD14 could be purified from myeloid cell culture supernatants and that the binding of AML-2-23 to myeloid cells was inhibited by soluble CD14 and by monoclonal antibodies to the soluble CD14 protein. These results suggested that a similar phenomenon might occur in vivo, a suggestion that was supported by the finding that soluble CD14 was present in normal human plasma and could be purified on AML-2-23 immunosorbent beads (*4*). A possible mechanism for the generation of a soluble CD14 peptide was subsequently provided by Haziot *et al.* (*5*), who demonstrated that CD14 is attached to the cell surface by a glycosylphosphatidyl-inositol linkage.

One hypothesis that emerges from these data is that soluble CD14 could be a natural inhibitor of the deleterious effects of endotoxin. LBP-LPS complexes could be neutralized by soluble CD14, which would prevent their interaction with macrophages and the induction of TNF release. This protective mechanism would be circumventedonce the levels of LBP-LPS exceeded the effective inhibitory concentration of soluble CD14. Thus, under normal conditions, soluble CD14 might neutralize the concentrations of LBP-LPS in a limited Gram-negative infection. Taken a step further, a process that triggers the release of CD14 from macrophages would enhance this protective effect in the face of a greater LBP-LPS load.

Clearly, additional experimentation is required to verify or disprove this hypothesis. Nevertheless, it suggests an obvious therapeutic application for recombinant soluble CD14 in Gram-negative sepsis.

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5 November 1990; accepted 21 March 1991

Response: Maliszewski correctly points out that CD14 can be found not only on the cell surface but also in culture supernatants of CD14-bearing cells and in human plasma (*1*). This observation was first made in 1985 and has been confirmed and extended (*2*). Because cell surface CD14 binds LPS-LBP complexes and appears crucial for initiating responses to LPS (*3*), Maliszewski hypothesizes that soluble CD14 may "neutralize" LPS-LBP complexes and thereby prevent responses to LPS. While this hypothesis is attractive on theoretical grounds, other observations suggest that soluble CD14 competes inefficiently with cell-bound CD14.

Bazil *et al.* (*2*) estimated the concentration of CD14 in plasma from healthy adults to be 2 to 6 μg/ml. This concentration ($\sim 10^{-7}$ M) is over a thousand times greater than the peak concentration of LPS (2×10^{-11} M) observed in human serum during sepsis (*4*). Thus, humans can respond to LPS briskly, and fatal-

Table 1. Loss of CD14 from monocyte-derived macrophages during response to LPS. The indicated stimuli were added to Teflon beakers containing 4-day cultures of human monocytes (10^6 cells per milliliter in RPMI, 10% normal human serum). After 18 hours of culture, cells were washed, stained with monoclonal antibodies to CD14 (3C10), CD18 (IB4), or HLA (W6/32) and fluoresceinated $F(ab)_2$ antimurine immunoglobulin G, and analyzed by FACS. Data are presented as mean fluorescent intensity. The loss of cell surface CD14 induced by LPS is unlikely to be secondary to the secretion of TNFα as addition of TNF enhanced, not decreased, the expression of CD14.

Antigen	Stimulant		
	None	LPS (100 ng/ml)	TNFα (10^{-9} M)
CD14	579	26	1138
CD18	1700	1636	1788
HLA	2292	2472	2399
Control	23	20	24

ly, in the presence of a molar excess of soluble CD14.

One explanation for this is that the CD14 could be damaged or inactivated upon release from cells. In any event, the absence of an effect of soluble CD14 at the relatively high concentrations present in plasma suggests that the released CD14 does not play an important role in blunting responses to LPS. The converse, however, may be true. Because cell-bound CD14 is important to a cell's response to LPS, depletion of CD14 from the cell surface through cleavage and release may effectively prevent a cell from responding to LPS. We have observed that stimulation of monocytes with LPS led to loss of surface CD14 (Table 1), which suggests a role for CD14 in the well-documented desensitization of leukocytes to the effects of LPS (*5*).

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4. In a recent study of septic patients, S. J. H. Van Deventer *et al.* [*Lancet* **i**, 605 (1988)] found maximum serum levels of 0.1 ng of LPS per milliliter ($<2 \times 10^{-11}$ M), while other patients with 20-fold less LPS were gravely ill. This is consistent with the finding of H. R. Michie *et al.* [*N. Engl. J. Med.* **318**, 1481 (1988)] that injection of LPS to $\sim 0.5 \times 10^{-11}$ M in healthy human volunteers initiates TNF release and fever.
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29 November 1990; accepted 21 March 1991

Receptor-Mediated Activation of Immunodeficiency Viruses in Viral Fusion

The mechanisms underlying human immunodeficiency virus (HIV) infection, thus far elusive, have important implications in the development of vaccines and therapeutics. The report by Moore *et al.* (*1*) provides convincing evidence that soluble receptor proteins block HIV infection by removing the gp120 protein from the viral envelope. They suggest that gp120 stripping may initiate viral fusion. We have found that simian immunodeficiency virus (SIVagm) infection was enhanced in the presence of soluble receptor protein (sCD4) and have proposed that sCD4 enhancement could be caused by the modulation of the viral envelope, resulting in the exposure of fusogenic domains that are involved in viral fusion (receptor-mediated activation) (*2*). Activation of the viral membrane through receptor binding has been reported for other fusogenic viruses (*3*) and may be a common mechanism in viral fusion.

In this light, we could not reconcile the blocking effects of sCD4 on HIV with our model. Moore *et al.* have provided an answer to these conflicting observations. In contrast to the activation of SIVagm by sCD4 that leads to viral enhancement, inactivation of the viral membrane by sCD4 in the case of HIV-1 would result in the premature loss of fusogenic sites. Although Moore *et al.* suggest that gp120 stripping may be a normal process in the initiation and exposure of fusogenic domains in viral entry, we believe that inactivation by sCD4 is an abnormal process and does not reflect events occurring at the cell surface.

To account for the enhanced infection of SIVagm by sCD4, we proposed (*2*) that association of CD4 with the oligomeric complex of gp120 molecules would expose fusogenic domains. This would be followed closely by viral fusion, presumably through a second receptor interaction with the exposed sites on the transmembrane protein. The intact oligomeric structure of gp120, in association with the transmembrane proteins, is probably essential in providing an environment for the correct orientation and exposure of fusogenic sites. Dissociation or stripping of gp120 from the viral membrane as it penetrates would be considered the last stage in viral fusion. If this model is correct, then the premature stripping effects observed in HIV would destroy the exposed fusogenic domains, which would inactivate HIV.

In the case of SIVagm, we hypothesize that a stronger association of gp120 with its transmembrane counterpart would allow for the activation by sCD4, which would result in enhanced viral infection through multiple sites on the virus. Stripping of gp120 by sCD4 in HIV would therefore not support viral infection because the integrity of the viral membrane would be lost. The final stage in viral entry would be artificially induced before the virus associates with the cell membrane.

In light of these findings, one should view therapies that involve receptor molecules with caution. It is possible that HIV strains may exist with more stable membranes. Therapeutic administration of sCD4 might activate more stable fusogenic domains on the virus, resulting in an enhanced rate of infection rather than viral inactivation.

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6 December 1990; accepted 21 March 1991

Response: Allan presents a model for sCD4-mediated enhancement of immunodeficiency virus-cell fusion (*1*). Enhancement was first observed for SIVagm infection of CD4-positive cells (*2*) and has since been demonstrated for HIV-2 (*3*). We have observed sCD4 enhancement of HIV-1 infection under certain conditions, but it is variable and incompletely characterized at present (*4*). A consequence of sCD4 binding to virions of HIV isolates adapted to tissue culture is the dissociation of gp120 from gp41 on the virion surface (*5–8*). We (*7*) and Allan *et al.* (*2*) hypothesized that sCD4 binding to SIVagm virions induces the viral envelope to expose cryptic fusogenic domains and that this enhances the efficiency of virus-cell fusion.

Conformational changes in the structure of gp120 or gp41 occur after CD4 binding. Thus sCD4 binding to gp120 increases the exposure of the V3 domain of gp120 to proteinases in vitro (*9*, *10*), and sCD4 binding to HIV-1 envelope-expressing cells induces conformational changes in gp120 that lead to increased exposure of gp41 epitopes (*6*, *10*). Changes in conformation can occur without complete dissociation of gp120 from gp41 (*10*). Therefore gp120 shedding may take place at a late stage of the fusion reaction (*1*) or may not occur at all at the cell surface. We suggest that an ordered sequence of conformational changes in gp120, gp41, and possibly CD4 (*11*) occurs during the fusion reaction. Perhaps this sequence involves cellular proteins in addition to CD4 (*9*, *12*).

Precisely how CD4 activates the fusion