Transfer of labeled oxygen from the oxidant to the carboxyl group of the product has been demonstrated by Sligar et al.[13c] Using $^{18}O_2$, we have found that ^{18}O is transferred to the carboxyl group of Leu-189 with ≈90% efficiency. In the crystal structure of HCAI, the 189-190 peptide bond is oriented parallel to Cys-212, while the 145-146 bond is directed away from it. The direct attack of coordinated oxygen on carboxyl carbon could be very sensitive to orientation, providing a plausible explanation for the observed selective cleavage. This suggests a cautionary note similar to what is observed in protein cross-linking: observation of the effect is good evidence for proximity, but lack of an effect may be due to unfavorable orientation rather than lack of proximity.

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Registry No. HCAI, 9001-03-0; Cys, 52-90-4; H_2O_2, 7722-84-1; ascorbate, 50-81-7.

Redirecting the Immune Response: Ligand-Mediated Immunogenicity

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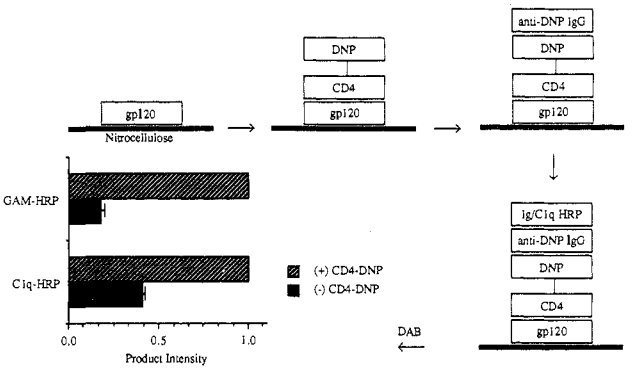
The immune system is remarkable in its ability to protect vertebrates from microorganisms. However, viruses and other pathogens have evolved a number of strategies for successfully evading neutralization by the immune system. For example, viral coat proteins can have high mutation rates,[1] conserved canyon-like motifs inaccessible to antibody combining sites,[2] self-like glycosylation patterns,[3] and only transient exposure of highly antigenic conserved sites involved in viral uncoating.[4] We now describe a new approach toward immunotherapy that may allow one to redirect the immune system's powerful memory response against substances not normally recognized as foreign. Immunological recognition of the target is achieved via a two-step process. Highly immunogenic antigens are tethered to ligands that bind selectively to conserved sites on a viral or cellular surface. Noncovalent binding interactions result in the introduction of this new antigen onto the target's surface, thereby tagging that microorganism or cell for recognition and neutralization by the immune system (Figure 1). This strategy suggests that it may be possible to direct a neutralizing antibody (ideally antibodies already present in sera[5-7]) against any cellular or viral surface for which a selective ligand is known. Such ligands might come from screens of recombinant libraries, collections, or fermentation broths.

This approach has been demonstrated in vitro by using the nonpeptidyl and peptidyl ligands, biotin and CD4, respectively, and the antigen, dinitrobenzene (DNP), which is recognized by approximately 1-2% of naturally occurring antibodies.[5] The CD4-DNP conjugate directs a monoclonal anti-DNP antibody to gp120, the envelope protein of the human immunodeficiency virus (HIV), via its functionally conserved CD4 binding domain.[8] The binding of soluble CD4 (sCD4) to gp120 has been extensively characterized (K_D = 10^{-9} M) and has been exploited in other anti-AIDS therapeutic strategies.[9-13] In the second example biotin directs the same anti-DNP antibody to the tetrameric protein streptavidin (K_D = 10^{-15} M). The antigen, DNP, elicits antibodies with 10^3-10^4-fold higher K_A's (2×10^8 M^{-1}) than do antigens of similar size.[14] In addition, natural anti-DNP antibodies account for 1% of all antibodies of the IgM subclass, and 0.8% of the IgG subclass with K_A's ≈ 10^4-10^6 M^{-1}.[15,16] In both examples the anti-DNP antibody is recognized by the first component of the complement cascade, C1q,[17] demonstrating the viability of ligand-mediated immunogenicity in generating active immunity against the target of interest, such as HIV or HIV-infected cells. In both cases the antigenic determinant only becomes multimeric and hence able to activate complement when presented in the context of the naturally occurring target protein.

Scheme I



Dinitrobenzene was derivatized with a water-soluble tetraethylene glycol spacer (16 Å) to ensure that the DNP group would be accessible for antibody binding. Monofunctionalization of tetraethylene glycol diamine with 2,4-dinitrofluorobenzene, followed by formation of the isothiocyanate with thiophosgene, afforded the acylating reagent which was used to introduce the DNP group onto the ϵ-amino groups on the surface of sCD4. Limited derivatization of the ϵ-amino groups of surface lysines on sCD4 resulted in a DNP conjugate that retained gp120 binding activity. The sCD4-DNP conjugate comigrated with unmodified sCD4 when analyzed by polyacrylamide gel electrophoresis with silver staining. The ultraviolet-visible spectrum of sCD4-DNP was consistent with 1 mol of DNP/mol of sCD4.

To test the ability of sCD4-DNP to target anti-DNP antibody to the HIV envelope protein gp120, an enzyme-linked immunosorbant assay (ELISA) experiment was performed (Scheme I). Recombinant gp120 was blotted onto nitrocellulose, and the remaining protein binding sites were blocked with bovine serum albumin (BSA). CD4-DNP was then added to the dot blot chamber and incubated for 30 min, and the excess CD4-DNP was removed by washing with PBS. Anti-DNP antibody AN09[18] was then added, followed by the same washing procedure. A second antibody, goat anti-mouse-Ig antibody-horseradish peroxidase (GAM-Ig-HRP), was used to detect the amount of anti-DNP antibody that bound gp120. Formation of the antibody complex was assayed by the addition of 3,3'-diaminobenzidine

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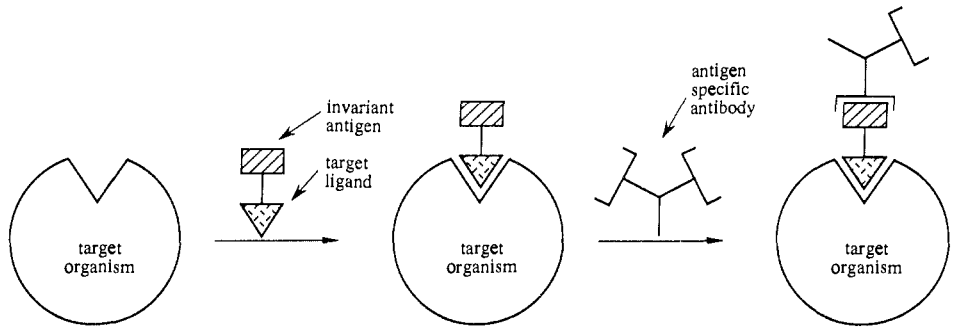
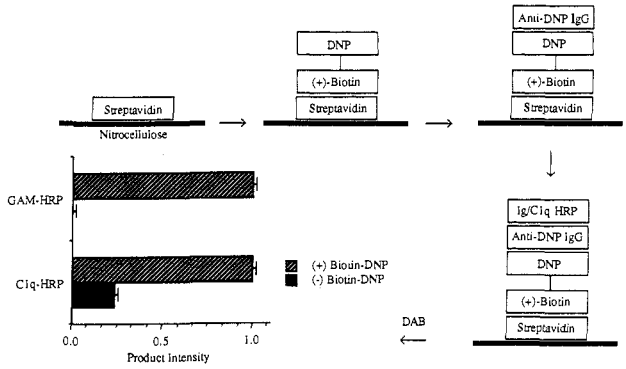


Figure 1. Schematic diagram of ligand-mediated immunogenicity.

Scheme II



tetrahydrochloride (DAB) and H_2O_2 (HRP substrates) at 4 °C and quantitated by densitometry of a positive exposure of the filter (Scheme I).[19] A control lacking CD4-DNP was performed to test for nonspecific binding of the anti-DNP antibody and the GAM-Ig-HRP conjugate. Controls lacking gp120 but containing CD4-DNP showed no signal (data not shown). In contrast, a large signal was obtained for the ELISA containing gp120, CD4-DNP, anti-DNP antibody, and GAM-Ig-HRP, indicating the successful targeting of antibodies to gp120 via noncovalently introduced epitopes.

The ability of the CD4-DNP gp120 complex to activate complement was assayed by substitution of C1q-HRP for GAM-Ig-HRP in the experiment described above. The first component of complement, C1q, is responsible for triggering the complement cascade to destroy cells on which immune complexes form.[17] C1q-HRP bound specifically relative to a control lacking CD4-DNP (Scheme I), demonstrating the successful formation of an immune complex on gp120, mediated by a noncovalently introduced epitope.[20]

In order to demonstrate the ability of small molecules to direct anti-DNP antibodies to proteins, a biotin-DNP linker was synthesized. Biotin-DNP was synthesized by condensation of DNP-tetraethylene glycol diamine and biotin using dicyclohexylcarbodiimide. Streptavidin was bound to nitrocellulose, and the sandwich assay was performed as described above (Scheme II).[21] Again the biotin-DNP conjugate successfully targeted anti-DNP antibody to streptavidin. A functional assay substituting C1q-HRP for GAM-Ig-HRP shows that the protein sandwich can also trigger a complement response. Streptavidin contains four biotin binding sites and thus may provide a better mimic of "capping" of antibodies on the surface of cells. Anti-DNP antibody also binds specifically to immobilized streptavidin in the presence of an excess of free biotin-DNP relative to the control lacking biotin-DNP.

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Supplementary Material Available: Full spectroscopic and analytical data for all compounds and experimental details (4 pages). Ordering information is given on any current masthead page.

(19) To a 3-mm-diameter circle of nitrocellulose was added 50 μL of gp120 (100 μg/mL) (30 min), and excess protein binding sites were blocked with a 3% BSA solution (2 h), followed by addition of CD4-DNP. (+) CD4-DNP = 100 μL (25 μg/mL) of CD4-DNP (30 min); (-) CD4-DNP = no CD4-DNP added. One hundred microliters (8 μg/mL) of anti-DNP antibody AN09 (30 min) was added followed by either 100 μL (1 μg/mL) of GAM-Ig-HRP (30 min) or 100 μL (10 μg/mL) of C1q-HRP (30 min). DAB substrate (4 °C) was added to the washed nitrocellulose. Product intensity was determined by a laser densitometer scan of a film positive of the nitrocellulose.

(20) The decrease in the ratio of specific to nonspecific binding of C1q-HRP compared to GAM-Ig-HRP is due to the low affinity of C1q for monomeric mouse IgG2a. C1q is hexameric and thus binds to aggregated IgG with great avidity ($K_A = 10^8\ M^{-1}$)[22] but binds monomeric IgG poorly ($K_A = 10^4\ M^{-1}$).[23] Because nitrocellulose is a solid support, the anti-DNP antibodies remain fixed rather than being able to aggregate or "cap" as they would on the surface of a cell. Goat anti-mouse-Ig antibodies typically have relatively high affinities ($K_A = 10^7$-$10^9\ M^{-1}$) for monomeric IgG and thus give a much better signal-to-noise ratio in this type of assay.

(21) Streptavidin (50 μL of 100 μg/mL) was substituted for gp120. Biotin-DNP (100 μL of 10 μg/mL) was substituted for CD4-DNP. GAM-Ig-HRP concentration was 2 μg/mL.

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Exploration of New Cooperative Proton-Electron Transfer (PET) Systems. First Example of Extended Conjugated Quinhydrones: 1,5-Dihalo-2,6-naphthoquinhydrones

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Quinones[2] have been utilized to produce novel charge-transfer (CT) complexes near the neutral-ionic interface.[3,4] Renewed

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