

Design, Production, and Testing

The compound used to selectively eliminate B cells is a 104.5 kd fusion protein consisting of a copy of gp120 — the targeting mechanism — attached to a toxin which will kill any cell that internalizes it.

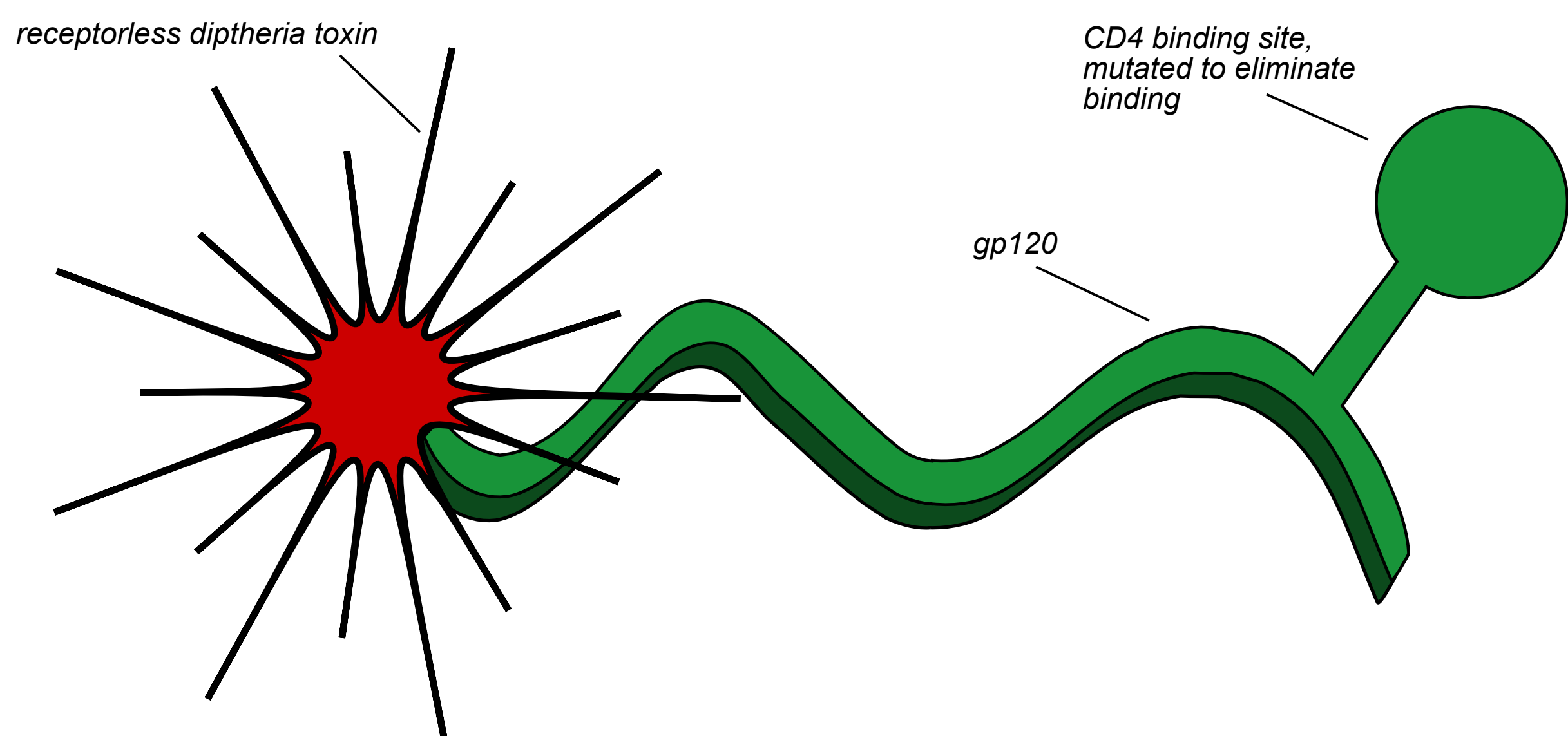
The design of this compound follows the methods of Ada and Byrt's work in

the late 1960s on B cell clonal selection, expansion, and memory, with one notable improvement: the choice of a toxin which is biologically acceptable in humans.

gp120 is bound to and internalized by only two kinds of cells in humans: the B cells which make antibodies against it and

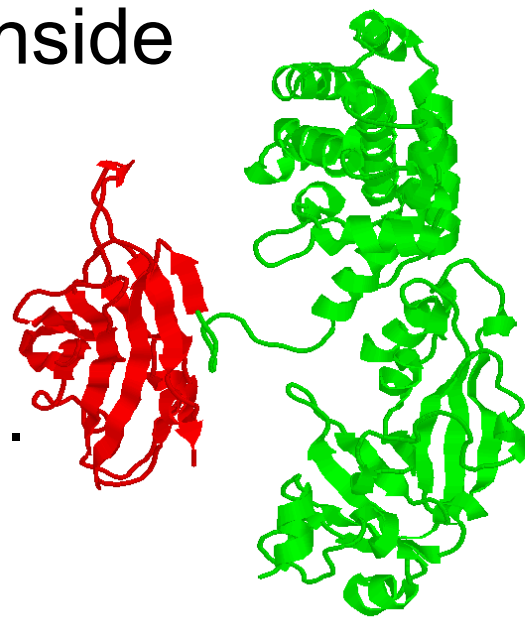
the CD4 receptors on T₄ cells. To prevent the drug from binding to and being internalized by T₄ cells, the CD4 binding site was mutated out. While gp120 is also bound by galactosylceramide, this binding does not result in internalization.

This protein drug can be produced easily in *E. coli* or dipthamide-negative cells. A C-terminal 6xHis tag allows the use of a simple, two-step purification process.



Receptorless Diphtheria

Diphtheria toxin contains three segments: the A chain, which is the active toxin, the B chain, which is the translocation domain, and the receptor binding segment. In diphtheria, the receptor binding segment, shown in red, is easily removed. Without the receptor, the toxin can not enter a cell, and it is essentially harmless. Once inside a cell, it kills at a concentration of only 1 or 2 molecules per cell.

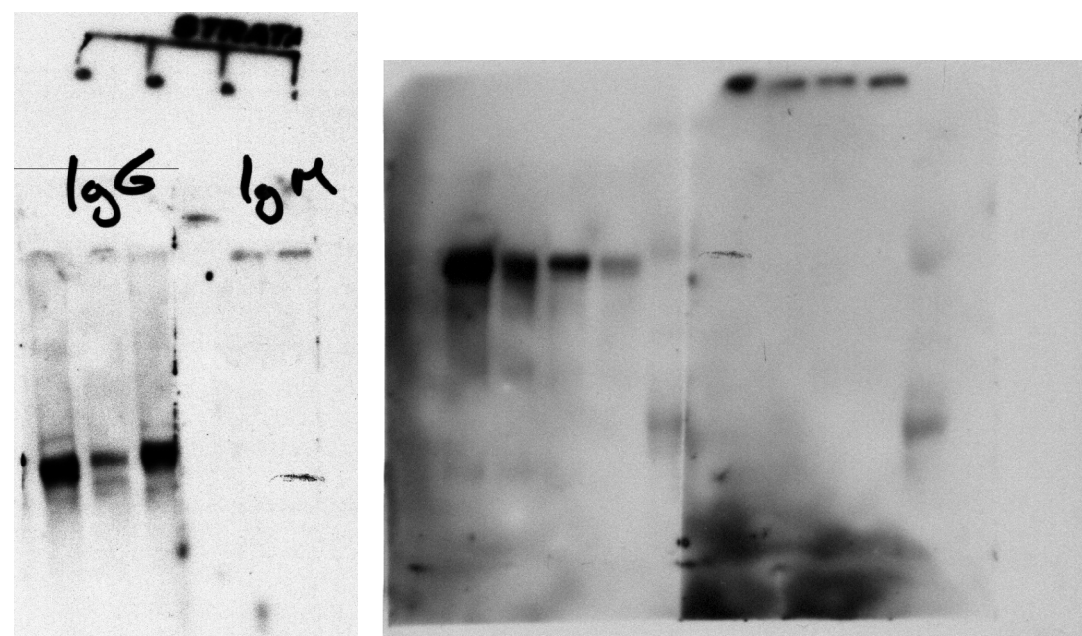


Eliminating CD4 Binding

gp120 has a high affinity for CD4 receptors, and is often internalized with CD4 by the T₄ cell it binds to. This is why gp120 concentrations are low early in HIV infections — which results in a slow antibody response. Internalization of this drug by any cell is lethal; to prevent it from binding to T₄ cells, two acidic amino acids in the CD4 binding site were replaced by two basic amino acids that preserve conformational epitopes while reducing binding by seven orders of magnitude.

Western Blot: Antibodies Reduced

Western blot test results show a significant reduction in gp120-reactive antibodies compared to controls without wholesale elimination of all antibodies. These results are consistent with previous studies following similar methods.



The first gel shows the results of a culture of PBMCs with gp120 peptide. Lanes 1 and 3 are controls; lane 2 was cultured with the drug. Note that in both gels, the results show total human antibodies, not just gp120 specific antibodies. The second gel shows that the drug does not indiscriminately eliminate all antibodies. The high antibody response seen in lanes 1 and 2 is the result of a culture of PBMCs with pokeweed mitogen (lane 1, control; lane 2, with drug). Lane 3 shows the antibody response to gp120 peptide without drug or pokeweed mitogen, and lane 4 shows the obviously lower level of antibodies seen with drug and peptide.

Vascular Leak Syndrome

Previous implementations of B cell clonal toxins used I-125 or plant toxins with non-specific activity, which are lethal in humans. Because of the known non-specific activity of other toxins, such as ricin, a culture of the drug with human vein endothelial cells was run to test for vascular leak syndrome — with clearly positive results.

Pilot *In Vivo* Safety Test

A small *in vivo* safety test, involving eight rats, was run with this drug to verify overall safety. Rats were injected with 4x and 8x expected monthly doses on a weight basis at 24-hour intervals. The blood counts (CBC with diff) and histopathology showed no detectable problems.

"The objective of this study was to determine if Immudel-gp120, a novel therapeutic designed to attenuate the action of HIV on the immune system, has any non-specific or unintentional side effects on the body. ... There were no significant or unusual findings upon necropsy of the animals in each of the groups. Histology on the tissues revealed no changes that could be attributed to the test article."

— SNBL Laboratories, summarizing the results of the pilot *in vivo* safety study