peptides of Busch and Gerdin (*26*) was not specified, but they may be polypeptides from the biologically active sites on fragment D.

Our results demonstrate that fibrinogen fragment D is an active principle in the specific disorganization of cultured vascular endothelial cells and suggest that fragment D plays a role in the pathogenesis of syndromes with vascular endothelial damage. Further investigations of fragment D with human capillary endothelial cells are necessary, as the pathological effects of fragment D may vary with the types and species of origin of the endothelial cells. The significance of fragment D in vivo and of its potential complex with fibrin monomer and fibrin fragment D dimer in the pathogenesis of vascular endothelial damage remain to be established.

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Protection from Genital Herpes Simplex Virus Type 2 Infection by Vaccination with Cloned Type 1 Glycoprotein D

Abstract. *Guinea pigs were vaccinated with truncated herpes simplex virus type-1 (HSV-1) glycoprotein D produced in the genetically engineered mammalian cell line gD10.2. Vaccinated animals formed antibodies that neutralized both HSV-1 and herpes simplex virus type 2 (HSV-2) in an in vitro neutralization assay. Vaccinated animals were challenged with HSV-2 by intravaginal infection. Animals that received the immunogen in Freund's complete adjuvant were completely protected from the clinical manifestations of genital HSV-2 infection. Animals that received the immunogen incorporated in alum adjuvants were partly protected from clinical disease; the infections that did develop were significantly less severe than those that occurred in control animals injected with adjuvant alone. The results demonstrate that immunization with a purified viral protein can provide significant protection against primary genital infection by HSV-2 in guinea pigs.*

The incidence of genital infections resulting from herpes simplex viruses (HSV's) has increased considerably over the past 20 years (*1*). Because HSV DNA is oncogenic in vitro (*2*), traditional methods of vaccine production must be approached with caution. Thus live attenuated virus vaccines, killed virus vaccines, and subunit vaccines consisting of viral proteins isolated from virus-infected cells are unlikely to gain acceptance until it can be shown that such preparations are free of potentially transforming fragments of viral DNA. Recent advances in recombinant DNA technology now make it possible to produce virtually unlimited quantities of purified viral antigens without resorting to large-scale cultivation of the infectious pathogen. Using this technology, we have attempted to develop an effective subunit vaccine to provide protection from primary infection by HSV type 1 (HSV-1) and HSV type 2 (HSV-2).

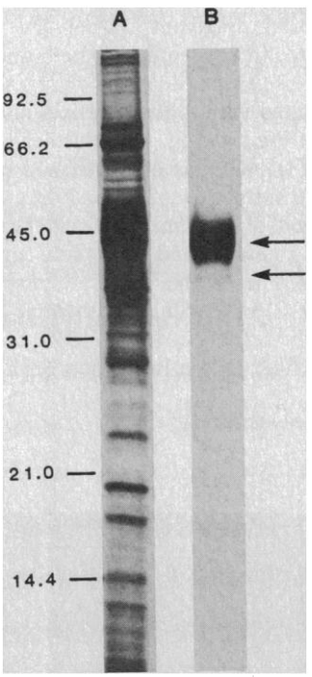

Fig. 1. Purification of truncated HSV-1 gD produced by the gD10.2 cell line. Serum-free culture medium conditioned by growth of the gD10.2 cell line was harvested and concentrated (*9*) and was further purified by affinity chromatography (*11*). Samples of the crude starting material and the purified product were analyzed on 10 percent polyacrylamide gels (*15*) and silver-stained. (A) Unfractionated culture medium from the gD10.2 cell line. (B) Purified gD-1 eluted from the affinity column. Arrows indicate the mature, fully glycosylated gD-1t (upper arrow) and the incompletely glycosylated gD-1t precursor (lower arrow). Mobilities (K) of molecular size markers are indicated in the left margin.

Both HSV-1 and HSV-2 are large enveloped DNA viruses that contain five or six major envelope glycoproteins, designated gA/B, gC, gD, gE, gF, and gG (*3*). In previous studies we (*4*) and others (*5*) cloned and determined the complete nucleotide sequence of the genes encoding glycoprotein D (gD) of HSV-1 and HSV-2. The sequence data confirmed immunologic data indicating that the two proteins were closely related (*6–7*) and suggested that antibodies elicited by either protein should cross-protect against HSV-1 and HSV-2 infections. In subsequent studies we demonstrated that both a full-length (*8*) and a truncated (*9*) gD-1 gene could be transferred into Chinese hamster ovary (CHO) cells and that permanent cell lines that constitutively synthesized a full-length, membrane-bound form of gD-1 and a truncated, secreted form of gD-1 (gD-1t) could be constructed. In addition, we found that vaccination of mice with gD-1t elicited an effective cross-neutralizing immune response that provided complete protection against a lethal intraperitoneal infection by HSV-1 or HSV-2.

Although the mouse provided a convenient model system in which to screen our early vaccine preparations, the guinea pig model more closely resembles human infections in that intravaginal infection of these animals with HSV-2 (*10*) leads to the development of acute vesi-

cles and ulcerations and can be followed by recurrent lesions and the establishment of ganglionic latency. In the study reported here we used the guinea pig model of genital HSV infection to test the efficacy of gD-1t as a subunit vaccine.

Subunit gD-1t was harvested from culture medium conditioned by growth of the gD10.2 cell line (*9*) and was purified from the concentrated cell culture medium by immunoaffinity chromatography (*11*). In Fig. 1 the composition of the material eluted from the affinity column is compared with that of the concentrated cell culture medium. The mature form of gD-1t (44K to 46K) is a major component of culture medium conditioned by the gD10.2 cell line (Fig. 1A). Fractionation of this material by immunoaffinity chromatography (Fig. 1B) resulted in considerable purification of gD-1t as well as of a precursor form of gD-1t (*9*) with a molecular weight of 39K. This purification scheme resulted in a preparation free of all contaminating proteins detectable by silver staining. To determine whether purification of the protein by this protocol denatured the protein or disrupted the antigenic structure of the molecule, antigenicity studies with a variety of monoclonal antibodies were conducted. In these studies we found that all antibodies tested, except those reactive with the carboxyl terminus, reacted with the purified preparation (*12*). No difference in antibody binding behavior could be detected with the purified preparation relative to the material found in unfractionated culture supernatants.

To examine the efficacy of gD-1t as a subunit vaccine to provide protection from primary genital infection by HSV-2, we vaccinated female Hartley guinea pigs with gD-1t formulated in various adjuvants. In the first experiment, purified gD-1t was incorporated in complete Freund's adjuvant (CFA) and injected at intramuscular and subcutaneous sites (*13*). Each animal received one primary immunization containing 30 μg of purified protein incorporated in CFA and, 31 days later, one booster immunization of the same amount of antigen incorporated in incomplete Freund's adjuvant (IFA). All animals were challenged by intravaginal inoculation of HSV-2 19 days after the booster immunization. Animals vaccinated with gD-1t produced high levels of antibodies that were capable of preventing both HSV-1 and HSV-2 virus infection in a virus neutralization assay in vitro (Table 1). Sera from these animals typically neutralized HSV-1 more effectively than HSV-2. This result is reasonable since the immunogen was derived from HSV-1 and since there are type-specific antigenic determinants on gD-1 (*6, 7*). More impressive was the finding that all the animals vaccinated with gD-1t were completely protected from the clinical manifestations of virus infection (redness, swelling, vesicle formation, ulceration, urinary incontinence, and lethal encephalitis) (Table 1). Of 14 animals injected with adjuvant alone, 13 developed severe primary infections. These results indicate that gD-1t incorporated in CFA can provide effective protection from genital HSV-2 infection.

Table 1. Protection of guinea pigs from genital HSV-2 infection by vaccination with recombinant HSV-1 gD.

Immunogen	Average neutralization titer in vitro*		Number of animals asymptomatic after HSV-2 challenge	Maximum mean lesion score†
	HSV-1	HSV-2		
gD-1t in Freund's adjuvant	9.3 ± 0.8	8.0 ± 1.4	15 of 15	0
Freund's adjuvant placebo	<3	<3	1 of 14	3.3 ± 0.4
gD-1t in alum-phosphate	9.2 ± 2.2	5.6 ± 2.2	2 of 9	1.3 ± 0.4
gD-1t in alum-hydroxide	10.9 ± 1.2	6.7 ± 1.5	4 of 9	0.9 ± 0.4
Alum-phosphate placebo	<3	<3	1 of 10	3.2 ± 0.4

*Neutralization titers were determined as described by Lasky *et al.* (*9*). Values represent the mean ± standard deviation of the highest dilution (log_2) of serum that inhibits virus infection. †The clinical symptoms of genital HSV-2 infection in guinea pigs were judged on a scale of 0 to 4, with 0 indicating no symptoms; 1, swelling and erythema; 2, small vesicles; 3, large vesicles; and 4, large, ulcerated lesions (*10*). Values represent the maximum mean lesion score (± standard error) within 30 days after challenge.

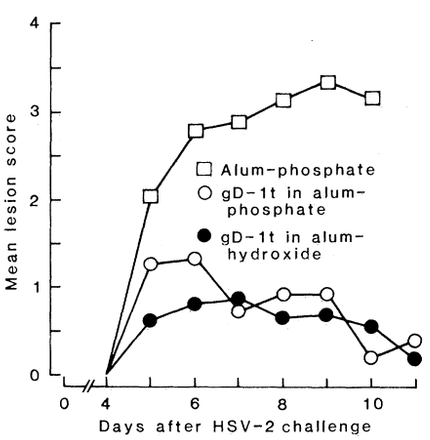

Fig. 2. Mean lesion scores in guinea pigs challenged by intravaginal infection with HSV-2 after vaccination with recombinant gD-1t. Female Hartley guinea pigs 2 months of age were divided into three groups (see Table 1) and immunized in accordance with a double-blind protocol with gD-1t in alum-phosphate, gD-1t in alum-hydroxide, or alum-phosphate alone (*13*). Twenty-seven days after the second booster immunization the animals were anesthetized with ketamine and xylazine and the intravaginal area was swabbed with 0.1*N* NaOH for 30 minutes; 0.1 ml of Dulbecco's modified Eagle medium containing 8000 plaque-forming units of HSV-2 (MS strain) was then instilled intravaginally with a blunted syringe needle. Challenged animals were observed for 1 month for the development of external signs of virus infection and were scored daily on a scale of 0 to 4 (see Table 1) (*10*). Animals with a score of 4 were killed 12 days after the viral challenge.

Because CFA is not acceptable for use in humans, we next wanted to determine whether gD-1t can protect against HSV-2 infection when formulated with an adjuvant suitable for human use. To this end, experiments with aluminum salt (alum)–precipitated protein complexes (*14*) were initiated. Guinea pigs were given three injections of gD-1t incorporated in the adjuvants alum-hydroxide or alum-phosphate or three injections of adjuvant alone (Table 1) (*13*). Both alum-based preparations elicited high levels of neutralizing antibodies against HSV-1, and the neutralizing titers against HSV-1 were comparable to those elicited against gD-1t incorporated in CFA. However, the titers of antibody capable of neutralizing HSV-2 were significantly lower with gD-1t incorporated in either of the alum preparations than with gD-1t incorporated in CFA. The findings suggest that incorporation of gD-1t in alum results in the loss of one or more antigenic determinants common to HSV-1 and HSV-2 and that alum-hydroxide is a more effective adjuvant than alum-phosphate [the neutralizing titers to HSV-1 and HSV-2 were significantly higher ($P = 0.03$, one-tailed Student's t-test), with the former than with the latter].

The difference in the HSV-2 neutralization titers obtained with the alum adjuvants relative to CFA was correlated with a reduction in the efficacy of the vaccine. Thus only 22 percent of the animals vaccinated with gD-1t in alum-phosphate and 44 percent of the animals vaccinated with gD-1t in alum-hydroxide showed no signs of virus infection. Although the protection provided by the alum adjuvant preparations was less effective than that obtained with the Freund's adjuvant preparations, it was still significant. While several animals showed signs of virus infection, the infections were considerably less severe than those observed in adjuvant-injected control animals [$P < 0.005$ (analysis of variance), beginning 5 days after chal-

lenge]. Thus maximum mean lesion scores were 1.3 in animals vaccinated with the alum-phosphate vaccine formulation and 0.9 in the alum-hydroxide formulation, compared to a maximum mean score of 3.3 for control animals (Table 1). Studies comparing different formulations and dosages of gD-1t are needed to determine whether the high level of protection achieved with CFA can be duplicated with an adjuvant acceptable for parenteral administration in humans.

Our results demonstrate that the clinical manifestations of primary genital HSV-2 infection can be significantly reduced by vaccination with recombinant gD-1t. It is not known whether these preparations were completely effective in preventing virus replication in the protected animals. Further studies will be required to determine whether vaccination against HSV with gD-1t prevents latent infection by the viruses or whether this type of vaccination merely diminishes the clinical symptoms of HSV infection. However, it is clear that a single HSV-1–derived glycoprotein can provide protection from the clinical symptoms of genital HSV-2 infection in the guinea pig when administered in conjunction with a potent adjuvant. Other studies will be necessary to determine whether HSV-2 gD or other HSV glycoproteins affect the potency of this vaccine. These considerations notwithstanding, we believe that our data justify further consideration of gD-1t as a subunit vaccine for prevention of HSV-1 and HSV-2 infections in humans.

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11. Truncated HSV-1 glycoprotein D was harvested from serum-free medium conditioned by growth of gD10.2 cells (*9*). The cell culture medium was clarified by filtration, concentrated by ultrafiltration, and fractionated by ammonium sulfate precipitation. The gD-1t was then purified to near homogeneity by immunoaffinity chromatography (T. Gregory, D. Vettlerlein, R. D. Hershberg, in preparation). The immunoaffinity column was prepared by coupling a monoclonal antibody produced against HSV-1 to cross-linked Sepharose CL-4B (Pharmacia Fine Chemicals) and eluted by a method similar to that described by R. Axén, J. Porath, and S. Ernback, [*Nature (London)* **214**, 1302 (1967)].
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13. Female Hartley guinea pigs 2 months of age and weighing approximately 250 g were purchased (Charles River). All the animals were immunized and scored in accordance with a double-blind protocol. For experiments involving Freund's adjuvant, the primary immunization consisted of gD-1t (30 μg) emulsified in 50 percent CFA, with 0.5 ml being injected subcutaneously into the loose skin above the neck and 0.5 ml injected intramuscularly into the thigh. After 31 days the animals were given the same amount of antigen incorporated in IFA. Control animals were injected by the same protocol as the experimentals, except that adjuvant alone was injected. Experimental and control animals were challenged intravaginally with HSV-2 19 days after the booster injections (see legend to Fig. 2). For experiments involving alum adjuvant, 30 μg of gD-1t incorporated in alum-phosphate or alum-hydroxide (0.15 ml) was used for both the primary and the booster immunizations. Protein in alum adjuvant was injected by intramuscular injection into the hind legs. Animals were given booster injections 25 and 51 days after the primary immunization and were challenged with live virus 27 days after the last booser injection.
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How Bees Remember Flower Shapes

Abstract. *Bees are able to learn to distinguish between flowers with different shapes or patterns. Some studies have suggested that bees remember only isolated features such as spatial frequency and line angles, rather than the photographic search images that are characteristic of vertebrates. New data indicate that this presumptive vertebrate-invertebrate dichotomy is false; bees can store flower patterns as a low-resolution eidetic image or photograph.*

One of the most interesting questions regarding learning is how information is processed and stored. Animals do not remember everything about a food source, for example, but focus on a particular and characteristic subset of cues. In many species, the same animal will concentrate on different constellations of cues in different behavioral contexts (*1*). A honey bee, for instance, can learn and remember a pattern of polarized light that it has seen in the sky and use the pattern in subsequent orientation, but the bee cannot learn the same pattern when it is offered as a cue for a food source (*2*). Animals frequently display strong spontaneous preferences within a sensory modality. Honey bees, for instance, prefer to land on and learn to recognize most quickly violet-colored food sources (*2*). Storage of what is remembered is not free of such biases. Honey bees store information about food sources in time-linked sets and must forget everything about a flower in order to learn a single change—that is, if only a flower's odor is altered, the bee must relearn its color, shape, and other characteristics even though these have not changed (*3*).

How honey bees remember a flower's shape—its outline and the pattern of colors on its petals is not known. Hertz (*4*) concluded that the bees' spontaneous preference for highly dissected patterns (shapes with a high ratio of edge to area, or high spatial frequency) was so great that only the crudest sort of learned discrimination was possible. Later, investigators (*5*) found that shape learning could be much more subtle, but concluded that spatial frequency and other, yet undefined and less important, characteristics were remembered rather than a photograph-like (eidetic) image. This is an attractive model: great neural economy is achieved (*3*), and the learning, which may resemble alpha-conditioning, can be rapid and reliable (*1*). However, Wehner (*6*), who showed that bees could learn the angle of a set of parallel lines on a vertical food source, initially concluded that an eidetic image was involved but later said that the bees might instead have been remembering the parameter of line angle as an isolated feature.

The main argument in favor of the proposal that bees remember a set of isolated features such as spatial frequency and line angle is their inability to generalize. Hence, bees that have been trained to feed on a black triangle do not select a triangular-shaped checkerboard pattern over, for instance, a square