oviduct (*11*) represents the only previously described role of progesterone in the regulation of synthesis of a specific protein. The selectivity of progesterone's action on milk protein synthesis may represent a greater sensitivity of the α-lactalbumin induction mechanism to progesterone, since at higher concentrations in vitro the induction of the galactosyltransferase is also inhibited. The inhibitory action of progesterone is manifested in vitro at concentrations which are nearly "physiological" for pregnancy (*8*), and lowering the progesterone concentration in vitro results in a release of its inhibitory effect. These observations are consistent with a proposed function of this hormone as a regulator of α-lactalbumin synthesis during pregnancy and lactation, but they do not suggest that progesterone regulates the onset or cessation of lactation itself. α-Lactalbumin acts as a "specifier" protein (*2*), interacting with the galactosyltransferase to cause inclusion of glucose as a substrate for galactosyl transfer. The hormonal regulation of product formation in this type of enzyme system may thus involve primarily a regulation of the induction of the specifier component.

ROGER W. TURKINGTON
ROBERT L. HILL

Departments of Medicine and *Biochemistry, Duke University Medical Center, Durham, North Carolina 27706*

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Molecular Heterogeneity of Human Lymphoid (HL-A) Alloantigens

Abstract. *Soluble preparations of HL-A alloantigens were separated by gel filtration into components having either the "LA" series or the "4" series of alloantigenic determinants. This separation may indicate that several different structural cistrons within the HL-A (locus) control the expression of these two series of determinants. The alternative possibility, in which one structural cistron with multiple mutational sites controls the synthesis of a single molecule cannot be excluded.*

We have reported the preparation of HL-A alloantigens in soluble form by papain treatment of human lymphoid cell membranes (*1*). At that time it was clear that the various products carrying alloantigenic specificities were not of the same size, because of the differences in their elution patterns from columns of Sephadex G-150. This observation has been pursued with more specific reagents detecting individual components of the "LA" and "4" series of alloantigens.

Soluble HL-A alloantigens were prepared by papain digestion of membrane extracts from human lymphoid tissue culture cells and spleen cells. Digestion mixtures were chromatographed on columns of Sephadex G-150, and the contents of individual tubes were concentrated to one-third the original volume and then tested for HL-A alloantigenic activity. A complement-dependent cytotoxicity test system was used, in which antiserums, known to detect different HL-A alloantigenic specificities, cause lysis and release of ^{51}Cr from target lymphocytes (*2*). The fractions from Sephadex G-150 were tested for their ability to inhibit the ^{51}Cr release. The reciprocal of the dilution of each fraction which gave a 50 percent inhibition of ^{51}Cr release was used to express the number of units of alloantigenic activity.

An example of a chromatogram of soluble HL-A alloantigenic material from a human lymphoid cell line (R-4265) is shown in Fig. 1. When the material from each tube was tested with various antiserums in the inhibition assay, four peaks of alloantigenic activity were found. Peak one had alloantigenic activity of the LA-2 and LA-4 determinants; peak two, the 6b and 7c determinants ("4" series); peak

Table 1. Chromatographic separation of HL-A alloantigens. Each peak listed corresponds to the region of the Sephadex chromatogram (Fig. 1) which shows HL-A alloantigenic inhibitory activity. Each region was tested for capacity to inhibit all antiserums shown, and only those serums are listed with which the alloantigens in that peak reacted. LA-1, -2, -3, -4, are alloantigenic specificities determined by the "LA" subdivision of the HL-A genetic locus, and 4a, 4d, 4c, 6b, 7c are antigenic specificities determined by the "4" subdivision.

Peak 1		Peak 2		Peak 3	
Specificities of LA	Antiserum	Specificities	Antiserum	Specificities	Antiserum
		*Tissue from R-4265**			
LA-2	Pinquette	4d, 6b	T-53		
LA-4	Jones	6b, 7c	Alvarez	LA-4	Jones
		4a	Kessler		
		4d, 6b	T-57		
		7c	Cutten		
		Tissue from spleen B			
LA-2	Pinquette	4d, 6b	T-53		
LA-3	Storm	4a	Kessler	LA-3	Storm
		7c	Cutten		
		4c	T-16		
		*Tissue from IM-1**			
LA-1	Morrison	4a	Kessler	LA-1	Morrison
LA-3	Storm	6b, 7c	Alvarez	LA-3	Storm
		4a	Buffo		
		*Tissue from RAJI**			
LA-3	Storm	4d, 6b	T-53	LA-3	Storm

* Human lymphoid tissue culture cell line.

three, the LA-4 determinants; and peak four, the determinants of both the "LA" series and "4" series.

These observations have been confirmed by chromatography of soluble HL-A alloantigens from three different tissue culture cell lines and a human spleen (Table 1). The "LA" series of alloantigens were regularly found in peaks one and three and were separated from the alloantigens of the "4" series which were found in peak two.

Several investigators have postulated that genetic regulation of the HL-A alloantigens is determined by at least two closely linked chromosomal regions within the HL-A locus (*3*). This hypothesis is based on serologic analysis of HL-A lymphocyte phenotypes and inferred genotypes in population and family studies. These studies have demonstrated two series of alloantigens ("LA" and "4") with multiple alleles in each series.

Independently and before reports of the genetic data, we made the initial observation reported in this paper, that is, that the two series of alloantigens when prepared by papain treatment of cell membranes are found in fractions separable by gel filtration.

If the HL-A alloantigens are primary gene products, two alternative hypotheses which are consistent with the genetic and molecular data and which provide a model for the structural gene control of their expression can be formulated. One structural cistron with two (or more) mutational sites ("LA" and "4") may give rise to a single molecular product which, when treated with papain, is released from the membrane, splits into two components, each of which carries either the "LA" or "4" determinant. Alternatively, two structural cistrons may code for separate molecules with the "LA" series or "4" series of HL-A alloantigens. Each would be released as a separate product after papain digestion, if this latter hypothesis is correct.

We believe that both hypotheses have precedence in human systems in view of prior experience with immunoglobulin G (IgG) and their Gm allotypes (*4*). Two separate allotypic regions [Gm(a) and Gm(z)] are found on a single $\gamma 1$ chain in the IgG1 subclass, probably reflecting control by one cistron with two mutational sites. After papain cleavage the Gm(a) determinant is found on the Fc portion of the molecule while the Gm(z) determinant is found on the Fab portion. If the HL-A alloantigens are similar and appear on a single molecule, papain treatment might yield two separate "pieces" from a primary molecule, each piece having either the "LA" or "4" series of alloantigenic determinants. This would be consistent with one structural cistron having two mutational sites controlling HL-A alloantigens.

When the Gm system was first identified, multiple complex alleles were postulated to account for the variety of allotypic patterns encountered in normal serum. Subsequently, the IgG1, IgG2, IgG3, and IgG4 subclasses of IgG were identified and shown to differ on the basis of their γ heavy chain components. Gm typing of representative IgG1, IgG2, IgG3, and IgG4 molecules showed that the alloantigenic determinants were restricted to one or another of the four subclasses. We now believe that the four subclasses of γ heavy chains are controlled by four separate cistrons. By analogy, separation of molecular forms of HL-A alloantigens which have the "LA" and "4" series of determinants may be consistent with two cistrons controlling these series of determinants.

Support for the hypothesis of multiple cistronic control in the HL-A histocompatibility system comes from a comparison to the mouse H-2 histocompatibility system. The H-2 genetic locus is divided into at least six subdivisions, five of which control the expression of different alloantigenic specificities found on cell membranes (*5*).

The sixth subdivision Ss, controlling the production of an apparently unrelated serum protein, has been shown by crossover studies to be within the H-2 locus between subdivisions which control the cell membrane associated alloantigenic determinants (*6*). Therefore it seems likely that at least two H-2 structural cistrons are located to either side of the Ss cistron. Consistent with the genetic map is the finding by Shimada and Nathenson (*7*) that Sephadex gel-filtration techniques separate papain-solubilized products from mouse lymphoid cell membranes

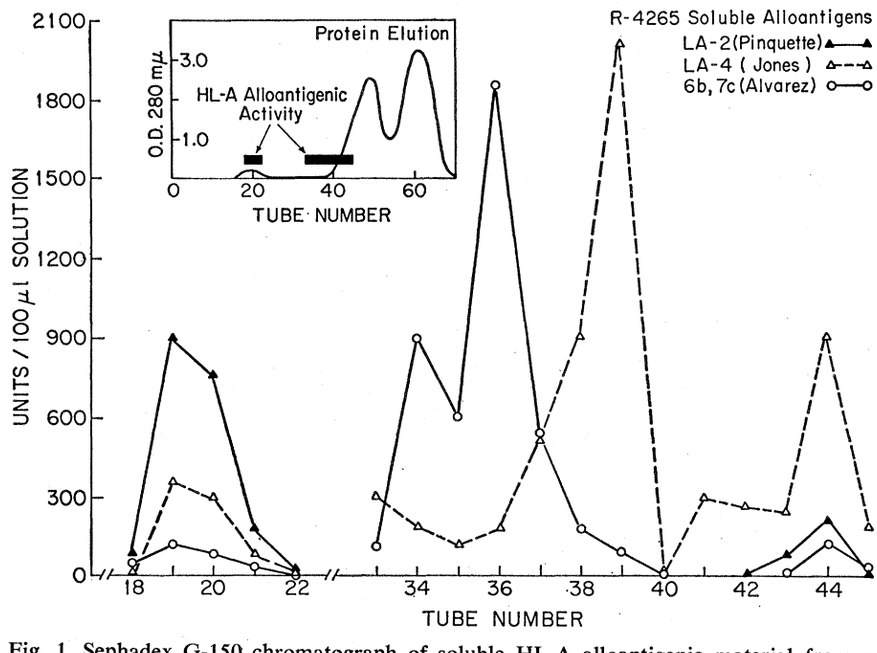

Fig. 1. Sephadex G-150 chromatograph of soluble HL-A alloantigenic material from a lymphoid tissue culture cell line (R-4265). The protein elution pattern is shown in the inset in the upper left. The HL-A alloantigenic activity (solid bars) was found in tubes 18 to 21 (excluded volume) and tubes 33 to 45 (included volume). The contents of individual tubes were tested for their relative inhibitory activity of various cytotoxic antiserums, and the results are expressed as units of activity on the vertical axis. Maximum inhibitory activity was found in four separate regions of the chromatogram and have been designated as activity peaks 1, 2, 3, and 4. Activity peak 1 represents inhibition of antiserums of the "LA" series. Pinquette (▲—▲), LA-2; and Jones (△---△), LA-4. Peak 2 represents the inhibition of an antiserum Alvarez (○—○) which recognizes the 6b, 7c determinants of the "4" series, and peak 3 the inhibition of the LA-4 antiserum, Jones. Peak 4 represents the inhibition of both "LA" and "4" antiserums.

into several sizes which carry different H-2 alloantigenic specificities. This separation is, in many respects, identical to the size separation of the HL-A alloantigen fragments.

The comparable molecular findings for the human HL-A alloantigens and the mouse H-2 alloantigens probably reflect a similar genetic mechanism, most likely that of multiple structural cistronic control.

DEAN L. MANN
G. NICHOLAS ROGENTINE, JR.
JOHN L. FAHEY
Immunology Branch, National Cancer Institute, Bethesda, Maryland
STANLEY G. NATHENSON
Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York

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Antifungal Steroid Glycoside from Sea Cucumber

Abstract. *An antifungal steroid glycoside, holotoxin, has been isolated from the sea cucumber* Stichopus japonicus *(Selenka). In vitro, it exhibits high activity against various fungi, including vegetable pathogens, but has scarcely any activity against Gram-positive and Gram-negative bacteria and mycobacteria in vitro.*

As a result of a study on the chemical components of certain species of sea cucumber, such as *Stichopus japonicus* (Selenka), *St. chloronotus* (Brandt), *Holothuria pervicax* (Selenka), *H. monacaria* (Lesson), *H. leucospilota* (Brandt), *Cucumaria frondosa* var. *japonica* (Semper) which inhabit the sea surrounding Japan, a new antifungal substance has been discovered. It has a high activity against pathogenic fungi and has been named holotoxin.

After the viscera and body fluid were removed, 1 kg of *Stichopus japonicus*, consisting of body wall tissues, was sliced and dried. To about 100 g of the dried material, 500 ml of methanol was added; the mixture was heated under reflux on a water bath for 6 hours and filtered while hot. This procedure was repeated three times with 300 ml of methanol. The filtrates were combined, and the methanol was evaporated under reduced pressure. The residue was dried and redissolved in 300 ml of hot methanol. The methanol solution was filtered to remove insoluble substances, and the methanol was evaporated under reduced pressure. The residue was stirred with 100 ml of benzene, and the insoluble material was recovered by centrifugation. The residue then was treated with 50 ml of benzene, and the solids were recovered. The insoluble material was next stirred with the minimum quantity of water required to form a suspension, and the material insoluble in water, obtained by centrifugation of the suspension, was washed with a little water to give the crude holotoxin. The crude holotoxin was purified by recrystallization from ethanol several times (yield, 87 mg).

Table 1. Minimum inhibitory concentration of holotoxin. Controls without holotoxin were conducted in the same way as the experiments, and they showed no activity.

Organism	Inhibition effect (μg/ml)
Sabouraud agar, 28°C, 96 hours	
Trichophyton asteroides	6.25
T. rubrum	6.25–1.56
T. interdigitale	6.25–1.56
Sabouraud agar, 28°C, 39 and 63 hours	
Candida albicans	16.7
Torula utilis	2.78
Saccharomyces cerevisiae	2.78
Potato agar, 28°C, 39 and 63 hours	
Penicillium chrysogenum	16.7
Aspergillus niger	16.7
Fusarium lini	16.7
Gibberella saubinetii	16.7
Glomerella cingulata	16.7
Ophiobolus miyabeanus	16.7
Piricularia oryzae	2.78
Potato agar, 28°C, 111 hours	
Helminthosporium avenae	16.7

Holotoxin forms colorless needles (melting point 250°C, with decomposition) and shows the following elementary analysis. Found: C, 51.90 percent; H, 7.93 percent; it exhibits no absorption in the ultraviolet region; in the infrared spectrum it has bands at 1745 and 1640 cm^{-1}, indicative of a five-membered ring lactone and one double bond, respectively. It is estimated to be a steroid glycoside, because after acid hydrolysis the aglycon, which is soluble in chloroform, gave a positive Liebermann-Burchard color reaction, and sugars in aqueous solution were detected by a positive reaction to aniline-phosphoric acid reagent and through the reduction of silver oxide. The infrared spectrum of holotoxin is closely similar to that of holothurin, which is a steroid saponin isolated from the sea cucumber (*1*). Holothurin is a sulfate, whereas holotoxin is not. The glycosides including holothurin do not exhibit antifungal activity. To our knowledge, holotoxin is the first antifungal glycoside which has been isolated from the animal kingdom.

The antifungal activity of holotoxin was tested in vitro. Crystalline holotoxin was dissolved in a 17.5 percent aqueous solution of dimethylformamide to give a solution having a concentration of 2 mg/ml. The resulting solution was serially diluted with sterile water and then added to a series of agar culture plates, each of which had been inoculated with a different test organism. The minimum inhibitory concentration of holotoxin was determined (Table 1).

Holotoxin has high activity against various fungi in vitro, including pathogenic organisms of vegetable origin, but it has scarcely any activity against Gram-positive and Gram-negative bacteria and mycobacteria (*2*).

SHIGETOSHI SHIMADA
Shimada Pharmaceutical Institute, No. 4-123, Kohamanaka, Sumiyoshi-ku, Osaka, Japan

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2. The results of clinical tests with holotoxin against superficial dermatophytosis in Kyoto University, Kyushu University (Fukuoka), Juntendo University (Tokyo), and Kitano Hospital (Osaka) demonstrated that holotoxin produced some improvement of symptoms in 77 cases out of 87, and this is equivalent to 88.5 percent effectiveness. Almost no side effects were noted.

12 December 1968