Hereditary Globulin Factors and Immune Tolerance in Man

Abstract. *Eight nontransfused donors of antibodies against a hereditary γ-globulin factor lacked the factor, but each donor's mother had it. The probability that this is a chance observation is .00003. A ninth donor had been transfused, and she and her mother both lacked the factor. It is assumed that each nontransfused donor's antibody was formed against his mother's γ-globulin and that immune tolerance had not been induced.*

Codominant alleles at two loci (Gm and Inv) determine antigens on human 7S γ-globulin (*1*). The 7S γ-globulin present in the human fetus and infant is acquired from its mother. Hence the γ-globulin phenotypes (Gm and Inv) of mother and newborn child are identical (*1*, *2*). Only after the child has lost its mother's γ-globulin and acquired its own does the child's phenotype correspond to its genotype.

It is generally recognized that immune tolerance to an antigen may be acquired by a mammal which is exposed to the antigen before birth or shortly after birth (*3*). Therefore one might expect the child to have acquired tolerance to the Gm and Inv antigens present in the mother's γ-globulin. We have found that such tolerance may not be acquired.

In our hands about 10 percent of healthy donors have serum which, when diluted 1/8 in saline, will cause red blood cells coated with an incomplete anti-D to agglutinate (*4*). About 10 percent of these agglutinating sera have antibodies (*5*, *6*) which may be used to detect some one of the Gm and Inv factors. Such sera are referred to as SNagg sera (*7*). We have observed that SNagg sera occur with essentially the same frequency among infants under 1 year of age as among adults. Indeed, a SNagg serum has been found in a 7-week-old infant (*4*).

Allen and Kunkel (*6*) have shown that multiply transfused children may develop anti-γ globulin reagents (that is, SNagg sera), thus offering strong evidence that these reagents are antibodies. Their observation led Richard Rosenfield to suggest that we examine the relation between the globulin types of our SNagg donors and their mothers; the objective was to test whether the donors, who are negative for the globulin factors they detect, may have become immunized by their mothers' γ-globulin. We had not done this previously because we had assumed that the donors would have acquired, while *in utero*, immune tolerance to their mothers' γ-globulin.

We were able to study nine donors of SNagg serum. Eight had not received transfusions at any time prior to the discovery of the active sample. One donor [Taylor, anti-Gm (x)] had received three units of blood 5 years before the active sample was drawn.

In eight cases, the hereditary γ-globulin type of the mother was determined with respect to the factor detected by her child's serum. In the ninth case (Wils.) the donor's mother was dead but her father, a sib, and a maternal aunt were available for testing. Her father was negative for the factor [Gm (a)], but her brother and her maternal aunt were positive for it. It seems reasonable to assume that the deceased mother was also positive for it. In seven of the other eight cases the direct tests showed the mother to be positive for the factor detected by the child's reagent. The sole exception was the mother of the donor of the anti-Gm (x), who was Gm (x—). It is significant that this is the only donor who had received transfusions prior to the discovery of the active sample. It seems likely that the transfusions were the cause of her immunization (*6*). The data are summarized in Table 1, which also shows the approximate age of the donor when the first sample was drawn, his race, and the approximate frequencies of the factors in the appropriate population.

These mother-child pairs were selected via the child, who was negative for the factor. In each case in which the donor had not been transfused the mother was positive for it. The probability of observing this by chance may be computed as follows. A mother can have a child negative for a factor if she is heterozygous for it or if she does not carry it at all. If we call the allele causing the factor *A*, and represent all others by *B*, the mother must be *AB* or *BB*—that is, she has at least one *B* allele. If the donor has been immunized by an antigen not acquired from the mother, the mother's second allele may be *A* or *B*, with probabilities determined by the frequency of the alleles in the population. Thus if *p* equals the frequency of the allele leading to the factor (*A* in the present example), it is also the frequency with which the mother of a child lacking it will have it. Since each pair recorded in Table 1 is independent of all the others, the probability of simultaneously observing all of them is the product of their separate probabilities, or

$$(.30)^3(.52)(.15)^2(.16)(.64)$$

This product equals .00003. The answer to our question appears to be that the observation is unlikely to have arisen by chance. Hence it seems probable that these untransfused donors of SNagg reagents have become immunized to their mothers' γ-globulin and that immune tolerance has not resulted from the presence of the antigen in large quantity prior to and immediately after birth.

We do not know how soon after birth this antibody may arise. We have not detected a single agglutinating serum in any of 353 samples of cord blood (*1*), so the agglutinating factor is not likely to be frequent at birth. The earliest we have detected it is in a 7-week-old infant. It should be noted, however, that we have not examined serum from infants between birth and 7 weeks of age. The agglutinator is usually a 19S globulin (β_2M) directed against 7S gamma globulin. Since the newborn infant produces small amounts

Table 1. Donors of SNagg reagents, their race, the factors detected by the SNagg and the approximate frequency of the factor in the race of the donor.

Donor	Race*	Age (yr)	Factor detected	Approximate frequency of factor in the donor's race	Reference
Wils.	W	25	Gm (a)	.30	(*9*)
Scol.	W	22	Gm (a)	.30	
Ma.	W	23	Gm (a)	.30	
Fuller	N	7/12	Gm (b)†	.52	(*4*)
Bright.	N	8	Gm (c)	.15	(*10*)
Griff.	W	1.5	Gm (aw)‡	.15	(*4*)
Davis	N	4	Gm $\overline{(bw)}$	.16	(*11*)
Lucas	N	27	Inv $\overline{(b)}$	.64	(*12*)
Taylor	N	35	Gm (x)	.04	(*4*)

* W, white; N, Negro. † This reagent detects Gm (b) in whites, but only 79 percent of Gm (b+) Negroes are positive for it (*4*). ‡ This reagent detects the Gm (a) factor in whites but does not seem to detect it in Negroes, hence the symbol $\overline{(aw)}$ (*4*).

of β_2M globulin, it is possible that the antibody is present at birth but because of the low concentration of the β_2M is not detected by our methods (*8*).

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References and Notes

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Coding Ambiguity in Cell-Free Extracts of Chlamydomonas

Abstract. *A cell-free system that incorporates amino acids into polypeptide has been prepared from extracts of the alga* Chlamydomonas. *The addition of polyuridylic acid to this system stimulates the incorporation both of phenylalanine and of leucine.*

The discovery that polyuridylic acid (*1*) and other synthetic polyribonucleotides can direct the incorporation of amino acids into polypeptides in cell-free extracts of *Escherichia coli* (*2*) has provided a valuable tool for studies of the genetic code (*3*) as well as of the role of ribosomes in protein synthesis (*4*). Synthetic polyribonucleotides can also direct the incorporation of amino acids in cell-free extracts of animal cells (*5*).

We have prepared a cell-free system from extracts of exponentially growing cultures of *Chlamydomonas* which can incorporate amino acids into polypeptides. *Chlamydomonas* is a unicellular sexual microorganism, an alga, that combines the subcellular organization of cells of higher plants and animals (*6*) with the exponential growth of microbial populations. As a phytoflagellate in the group from which both the higher plants and the animals have evolved (*7*), *Chlamydomonas* is in a central phylogenetic position with respect to considerations of the universality of the genetic code. The organism is being used for studies of nucleic acid and protein synthesis, especially for comparison with bacterial and mammalian cells (*8*).

The organism (strain 21gr) was grown on a minimal culture medium (*8*). Cultures were grown from log-phase inocula for four to eight generations with a constant 6-hour doubling time. Under these conditions, the concentrations of RNA and protein per cell remain constant until the culture reaches a density of about 5×10^6 cells per milliliter (*8*). In these experiments, cells were harvested when growth reached 2 to 3×10^6 cells per milliliter, washed with standard buffer (tris-HCl, *p*H 7.8, $10^{-2}M$; magnesium acetate, $10^{-2}M$; KCl, $6 \times 10^{-2}M$; mercaptoethanol, $6 \times 10^{-3}M$) (*2*), and resuspended in 5-ml total volume per gram, wet weight, of cells. The harvesting and all subsequent preparative steps were carried out at 2°C. The cells were broken with a Mullard ultrasonic oscillator (10 kv) for 3 minutes (95 to 99 percent breakage). The broken-cell suspension was centrifuged at 10,000*g* for 10 minutes and the supernatant fluid was recentrifuged at 30,000*g* for 30 minutes. The resulting supernatant fraction (S-30) was separated into a ribosomal and supernatant fraction (S-100) by centrifugation for 2 hours at 105,000*g*. The pellet was suspended in standard buffer that also contained 0.1 percent DOC and was resedimented at 105,000*g* for 2 hours. The supernatant fluid was discarded, the tube was rinsed with 2 ml of buffer, and the pellet was resuspended in one-tenth of the original volume of the same buffer. Soluble RNA was prepared by phenol extraction (*5*) of the S-100 fraction. The S-30, S-100, and DOC ribosome fractions were prepared on the same day on which they were used for incorporation studies. The reagents used, as well as the procedures for incubation, precipitation, and radioactive counting were those of Nirenberg and Matthei (*2*) as modified by Weinstein and Schechter (*5*). The composition of the reaction mixture is given in Table 1.

The extent of incorporation of C^{14}-phenylalanine into polypeptide by cell-free extracts of *Chlamydomonas* is shown in Table 1. The S-30 fraction had a low endogenous activity, but the addition of 100 μg of polyU to this system resulted in a 31-fold stimulation of phenylalanine incorporation. Supplementing the system with sRNA from *Chlamydomonas* increased this incorporation from 31-fold to about 61-fold. A comparable enhancement of the polyU stimulation was also obtained

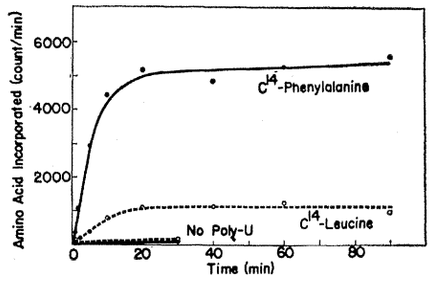

Fig. 1. Rate of polyU-stimulated phenylalanine and leucine incorporation in the *Chlamydomonas* system. The reaction mixture is as described in Table 3. Reactions were stopped at the indicated times by addition of TCA.

Table 1. Polyuridylic acid stimulation of C^{14}-phenylalanine incorporation by cell-free fractions of *Chlamydomonas*. When polyU was present in the reaction it was added in 100 μg amounts. The reaction mixture contained the following (in micromoles unless otherwise specified): tris-HCl buffer, *p*H 7.8, 2.3; magnesium acetate, 2.3; KCl 13.8; mercaptoethanol, 1.3; ATP, 0.25; PEP, 1.25; GTP, 0.01; CTP, 0.01; UTP, 0.01; PEP kinase, 12 μg; C^{14}-phenylalanine, 0.8 (160 mc/mole); and a mixture of 19 C^{12} amino acids excluding phenylalanine, 0.0125 each. The other components are specified in the table. The total volume of the reaction mixture was 0.3 ml. Tubes were incubated 40 min at 35°C. The reaction was stopped with TCA. The product was prepared for counting (*5*). Contents of ribosomal protein in the fractions were 87 μg in the S-30 and 62 μg in the ribosomes. The S-100 contained 0.31 μg protein and was prepared by two successive centrifugations of the supernatant at 105,000*g* for 2 hours to insure against contamination by ribosomes.

Cell fraction	Additions	C^{14}-phenylalanine incorporated	
		Radioactivity (count/min)	Ribosomal protein (mμmole/mg)
S-30	None	71	0.15
S-30	PolyU	2239	4.7
S-30	PolyU; 42 μg sRNA *	4222	8.9
Ribosomes	PolyU	0	0
S-100	PolyU	0	0
Ribosomes plus S-100	PolyU; 42 μg sRNA *	1346	4.0

* From *Chlamydomonas*.