antigenic determinant of the pneumococcal C polysaccharide. The present system is, therefore, unique in that a primary PFC response to a well-defined hapten, phosphorylcholine, is obtained by immunization with a complex antigen, strain R36A pneumococci.

The complete inhibition of primary plaque formation observed with anti-idiotypic serum against either TEPC-15 or HOPC-8 myeloma proteins supports the following conclusions: (i) the idiotypic determinants of immunoglobulin molecules are specific markers probably located in the variable region of the molecule and might be in the vicinity of the antigen binding site *(15)*; (ii) the TEPC-15 and HOPC-8 myeloma proteins and the antibodies to R36A pneumococci must share very similar or identical idiotypic determinants; (iii) primary immunization with strain R36A pneumococci elicits an antibody response which is restricted in regard to idiotypic determinants; and (iv) similar idiotypic determinants are carried across immunoglobulin classes since anti-idiotypic serums to IgA myelomas also recognize the same determinants on antibodies of the IgM class (direct plaque-forming antibodies). We believe that the latter finding supports the hypothesis of a common gene pool for the variable heavy chain region (V_H) which is shared by the major immunoglobulin classes *(16)*.

Presumably, immunocompetent cells specifically reactive with strain R36A pneumococci have on their surface receptors for phosphorylcholine. If the combining site of the receptor is similar or identical to the binding site of the naturally induced antibodies to phosphorylcholine then anti-idiotypic serums should provide an effective probe for the immunological function of the antigen cell receptor. Indeed, some experiments indicate that anti-idiotypic serums specifically prevent the induction of the primary response to phosphorylcholine in vitro *(17)*. With the availability of anti-idiotypic serums, phosphorylcholine antiserums, and free phosphorylcholine, it should be possible to dissect many of the complex interactions between cell types, cell receptors, and the hapten-carrier during the course of the immune response.

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8. At day 4 after immunization spleen cells from mice immunized with SRBC were also mixed 1 : 1 with spleen cells from mice immunized with heat-killed pneumococci. This spleen cell mixture was plated against SRBC, and the slides were incubated in Hanks balanced salt solution (H-BSS) or in a 1 : 1000 dilution of anti-idiotypic serum before addition of GPC. The number of plaques developed against SRBC in both sets of slides was similar, an indication that the reaction of anti-idiotypic serum and antibody to pneumococci was not binding all of the complement.
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11. Inhibition of plaque formation was just as effective if the slides containing the spleen cells, from animals immunized with pneumococci or from cultures, were first incubated in H-BSS for 1 hour before incubation in phosphorylcholine or anti-idiotypic serum for 1 hour and addition of GPC. Thus, it appears that the inhibitors interact with the antibody synthesized by the spleen cells and prevent it from binding to the target cells. The possibility is not excluded, however, that inhibition of plaques by anti-idiotypic serum could be caused by either conformational changes of the pneumococcal antibodies, thus indirectly affecting the binding of complement.
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Biosynthesis of C4 (Fourth Component of Complement) by Hybrids of C4-Deficient Guinea Pig Cells and HeLa Cells

Abstract. *Peritoneal exudate cells from guinea pigs homozygous for a genetic deficiency of the fourth component of complement (C4) were fused in vitro with a cell line of human origin (HeLa). The resulting hybrid cells, derived from cell lines each incapable of C4 synthesis by themselves, synthesized functionally active human C4.*

The serum complement system consists of nine distinct proteins that interact to mediate and amplify many of the biological effects of antigen-antibody reactions. A number of genetic deficiencies of serum complement (C) have been described in man *(1)* and experimental animals *(2)*. Among these is an autosomal recessive trait in guinea pigs which, in the homozygote, results in a total deficiency of the fourth (C4) component of complement *(2)*. Evidence has been presented that peritoneal exudate (PE) cells isolated from homozygous C4-deficient animals were capable of synthesizing C2 (the second component of complement) for more than 5 weeks in tissue culture, but did not produce any detectable C4 *(3)*. In contrast, under the same conditions, normal guinea pig PE cells were capable of synthesizing both C2 and C4 *(3)*. In an attempt to define the genetic lesion responsible for this inherited deficiency in C4 biosynthesis, PE cells from a homozygous C4-deficient guinea pig were fused in vitro with a cell line of human origin (HeLa). The resulting hybrid cells, derived from cell lines

Table 1. In vitro synthesis of C4 and C2 by HeLa, C4-deficient, and hybrid cell lines.

Cell line	Effective molecules (10^{-8}/dish)*	
	C4	C2
Hybrid (DP)	13.1	0.0
Hybrid (D3)	15.6	0.0
Hybrid (D11)	0.0	0.0
HeLa	0.0	0.0
C4-deficient PE†	0.0	20.7

* Limits of detection, 1×10^7 effective molecules per milliliter. † Assayed in primary culture 4 weeks after isolation.

that were incapable of C4 synthesis by themselves, synthesized functionally active human C4.

Starch-induced PE cells were obtained from a homozygous C4-deficient animal (*3*). The cells were washed three times in medium 199 (M199, Microbiological Associates) at 10°C; then once in Hanks balanced salt solution (HBSS). The PE cells were resuspended in HBSS at a concentration of 1×10^6 cell/ml and mixed with 1×10^6 glutamine-dependent HeLa cells (*4*) in a total volume of 2 ml. The cell mixtures were incubated for 45 minutes at 0°C to allow spontaneous fusions between PE and HeLa cells. The cells were then transferred to petri dishes (60 by 15 mm, Falcon Plastics), and cultured in complete Dulbecco's medium with glutamine ($2 \times 10^{-3}M$) and 10 percent fetal calf serum (FCS) (*5*) at 37°C in a humidified atmosphere consisting of 5 percent CO_2 and 95 percent air. After 1 day, the medium was changed to Ham's F-12 containing glutamic acid ($1 \times 10^{-3}M$) and 15 percent dialyzed FCS (GA medium) (*5*). The GA medium selects for hybrid cells because neither glutamine-dependent HeLa cells nor guinea pig PE cells will propagate in this medium. Hybrid cell colonies were isolated and allowed to grow to confluence in separate dishes (60 by 50 mm). Approximately 6½ weeks after hybridization in vitro, the GA medium was replaced with M199 supplemented with 10 percent FCS that had been heated to 56°C for 2 hours. After 72 hours, the tissue culture media were removed and assayed for hemolytically active C4 and C2 (*6*). The cells were then incubated in 3 ml of M199-FCS in which a mixture of ^{14}C-labeled leucine, lysine, isoleucine, and valine (final concentration 3 μc/ml) were substituted for the corresponding unlabeled amino acids. After incubation for 72 hours at 37°C, the medium was removed, dialyzed exhaustively against barbital-buffered saline (ionic strength, 0.15; *p*H 7.35), then concentrated to approximately one-tenth its volume. A portion of the concentrated medium was then mixed with normal human serum, and another portion with normal guinea pig serum. These mixtures were then placed in wells cut in 1 percent agarose opposite specific antiserums to human C4 and to guinea pig C4 (*7*). The immunodiffusion slides were incubated for 72 hours at 4°C, then washed first in 0.15*M* NaCl, and then in H_2O, dried, and stained. The dried slides were then exposed to Kodak x-ray film for 8 weeks to detect incorporation of radiolabeled amino acids into C4 protein. Chromosome preparations of the hybrid cells were made as described (*8*). The presence of guinea pig antigens on the cell surface of hybrid cells was detected by a modification of a fibroblast cytotoxic assay (*9*). Briefly, HeLa, C4-deficient fibroblasts, and hybrid cells were each

Table 2. Species-specific functional properties of C4 synthesized by hybrid cells.

Converting reagent	Effective C4/cell		Effective C4 (10^{-8}/dish) D3-hybrid cells
	Guinea pig serum*	Human C4†	
Human C2	0.190	1.509	10.9
Guinea pig C2	1.871	1.419	9.9

* 1/300,000 dilution of guinea pig serum. † 1/1000 dilution of partially purified human C4 (Cordis).

incubated for 1 hour at room temperature with rabbit antiserum to guinea pig tissue (*10*), and then for 1 hour at 37°C with undiluted fresh guinea pig complement. Cytotoxicity was estimated by trypan blue dye uptake.

The results of these experiments (Table 1), indicated that some of the hybrid clones and the uncloned hybrid cell population were capable of synthesizing hemolytically active C4. HeLa cells and C4-deficient PE cells alone produced no detectable C4 under the same conditions. Furthermore, by immunochemical analysis of the ^{14}C-labeled C4 (Fig. 1) and by its ability to interact with human C2 (Table 2), the C4 produced by the hybrid cells was identified as human in origin (*11*).

The following data established that the cells producing C4 were indeed human–guinea pig hybrids. (i) Antiserum to guinea pig serum and guinea pig complement effected lysis of more than 90 percent of the hybrid cells (D3) and more than 90 percent of a C4-deficient fibroblast cell line. Less than 5 percent of HeLa cells were lysed under the same conditions. (ii) A karyotype of the hybrid cells (Fig. 2) showed both human and guinea

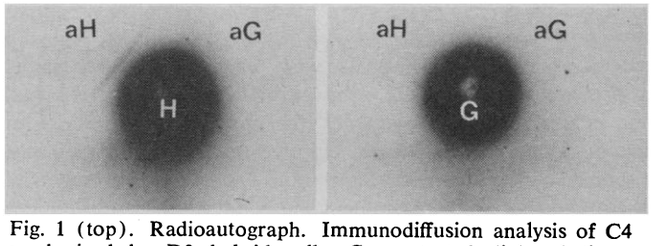
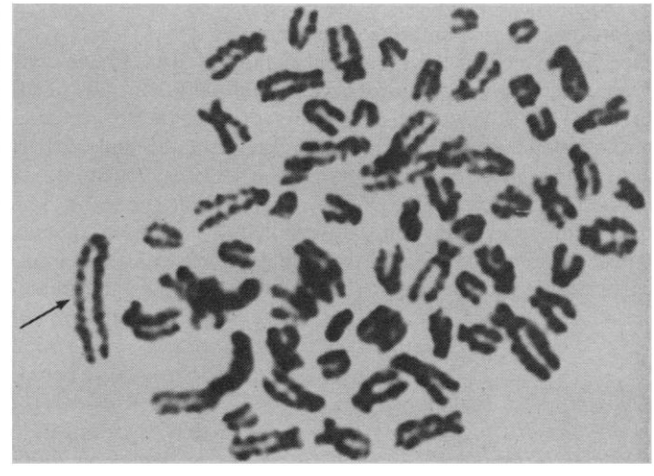


Fig. 1 (top). Radioautograph. Immunodiffusion analysis of C4 synthesized by D3 hybrid cells. Concentrated dialyzed tissue culture medium mixed with normal guinea pig serum (*G*), and with normal human serum (*H*). Antiserum to human C4 (*aH*), and antiserum to guinea pig C4 (*aG*). Guinea pig and human serums were included to provide carrier protein for immunoprecipitation. Fig. 2 (right). Metaphase plate of hybrid of C4-deficient guinea pig cells and HeLa cells (D3). Length of the long arms of acrocentric chromosome (indicated by arrow) identifies this chromosome as of guinea pig origin. The appearance of a normal guinea pig karyotype is given in (*13*).



pig chromosomes. Significantly, very few guinea pig chromosomes were visible in these preparations and the human chromosome content of the hybrids was somewhat less than that of the HeLa cell line.

Two principal interpretations of these data were considered. (i) The genetic information derived from C4-deficient guinea pig cells may have been capable of initiating C4 synthesis by cells (HeLa) in which this function had not previously been expressed. This would indicate that the genetic abnormality responsible for C4 deficiency is probably not a result of repression of gene function, but rather is a consequence of an inability to respond to an initiator signal, that is, perhaps due to a structural gene abnormality. (ii) Alternatively, it is possible that fusion of guinea pig and HeLa cells led to the loss of a chromosome bearing information for the repression of C4 synthesis, but not loss of the chromosome bearing the C4 gene. A loss of repressor might then permit expression of human C4 gene function. It is not yet possible to distinguish between these two alternatives.

Levy and Ladda (*12*) have obtained indirect evidence that genetic information required for the production of C5 could be incorporated into cells obtained from C5-deficient mice. These cells, hybrids of C5-deficient cells with chicken erythrocytes, when administered to the deficient mice, led to a temporary restoration of hemolytic complement activity in the serums of the recipients. Unfortunately, the gene product (C5) was not directly identified, nor was it possible to determine whether genetic information derived from the deficient or the intact cell (chicken erythrocyte) was directing the synthesis of C5. Our experiments permit identification of the gene product (C4), as well as the functional and antigenic species-specific characteristics of the C4. A definition of the precise genetic lesion responsible for inherited deficiencies of plasma proteins requires specific identification of the gene product. Ultimately, correction of the genetic abnormality would then be possible.

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Carbonic Anhydrase and Bone Remodeling: Sulfonamide Inhibition of Bone Resorption in Organ Culture

Abstract. *Five sulfonamide inhibitors of carbonic anhydrase inhibited parathyroid hormone–induced resorption of bone in organ culture. The relative activities of the sulfonamides as resorption inhibitors were such as to suggest the presence of a functional carbonic anhydrase system in bone linked to the mechanism of bone resorption.*

The enzyme carbonic anhydrase (CA) is widely distributed in nature. In mammals it serves two general physiological roles. The first is in respiration, where it functions in erythrocytes to catalyze the uptake and release of carbon dioxide. The other function is in various secretory organs (for example, kidney, stomach, pancreas, ciliary process), where it is involved with the

Table 1. The effect of sulfonamide inhibitors of carbonic anhydrase on PTH-induced resorption in organ cultures of bone. Resorption was assessed by measuring the transfer of ^{45}Ca from bone to medium. Percentage inhibition was calculated by using the formula $(P - I/Pc) \times 100$, where P and I represent the radioactivity found in culture mediums treated with PTH (P) and PTH plus inhibitor (I), respectively. The denominator (Pc) estimates the isotope found in the medium of PTH-treated cultures due to cell-mediated activity corrected for physicochemical exchange and crystal dissolution as discussed previously (*8*). The mean and standard error of the mean of four to eight observations are given in column three.

Inhibitor	Molar concentration	Percent inhibition of PTH-induced resorption
Sulfanilamide	1×10^{-2}	68 ± 4
	1×10^{-3}	35 ± 3
	1×10^{-4}	1 ± 8
Acetazolamide	4.5×10^{-4}	98 ± 1
	1×10^{-4}	92 ± 3
	4.5×10^{-5}	59 ± 2
	1×10^{-5}	17 ± 5
	4.5×10^{-6}	0
Methazolamide	1×10^{-3}	96 ± 2
	1×10^{-4}	87 ± 4
	1×10^{-5}	29 ± 4
	1×10^{-6}	9 ± 4
Benzolamide	1×10^{-3}	93 ± 2
	1×10^{-4}	49 ± 6
	1×10^{-5}	6 ± 1
Ethoxzolamide	1×10^{-5}	78 ± 3
	2.5×10^{-6}	34 ± 2
	1×10^{-6}	5 ± 3
	1×10^{-7}	12 ± 1