Our findings provide possible practical implications for pairing of donor and recipient for transplantation. Genetic differences exist in the MHC, which are very difficult to detect serologically even though they can be the cause of rapid rejection of skin.

Although some studies have yielded evidence suggesting a correlation between pairing by typing for the HL-A antigens in man and graft survival (better survival given fewer antigenic differences), these studies have been somewhat disappointing (*21*). Similarly, the cells of a majority of unrelated individuals who appear to have identical SD antigens stimulate in MLC tests (*22*). Many possible reasons have been given for the lack of strong correlations; heterogeneity of the HL-A antigens, polyspecificity of the available serums, and our lack of knowledge concerning the strength of the different antigens have all been mentioned. Our findings suggest that one reason for the lack of a strong correlation may be the presence of LD loci—serologically undetected differences that can lead to MLC activation and skin graft rejection.

The H(z1) mutant suggests that in at least some cases the same mutation can affect both skin graft rejection and MLC activation; although one cannot be certain that only one gene is involved in this mutational event. While some LD differences may lead to skin graft rejection, others, such as the 4R-2R, do not. In the 4R-2R combination there is stimulation in only one direction which is a very unusual finding using normal allogeneic cells; further experiments will be needed to study the relation of graft rejection to LD differences and MLC activation. At the present time studies with MLC tests, serological detection of antigens, and skin graft studies (where possible) are needed to gain a more complete understanding of the MHC, the factors important for MLC activation and those important for graft survival.

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Specific Inhibition of Plaque Formation to Phosphorylcholine by Antibody against Antibody

Abstract. *Spleen cells from BALB/c mice immunized with heat-killed rough pneumococci (strain R36A) or spleen cells from normal mice immunized in vitro with the same antigen produce direct hemolytic plaques against sheep erythrocytes coated with pneumococcal C polysaccharide or conjugated with phosphorylcholine. Formation of plaques is specifically inhibited by phosphorylcholine or by antiserum to mouse immunoglobulin A myeloma protein which binds phosphorylcholine. Thus, the myeloma proteins and normal BALB/c antibodies share similar idiotypic determinants. This experimental system is suitable for probing the role of the antigen receptor in the immune response.*

Myeloma proteins obtained from mice carrying plasma cell tumors have detectable antibody activity against chemically defined haptens such as phosphorylcholine (*1*). Potter and Lieberman have reported the preparation of antiserums to idiotypic determinants in BALB/c myeloma proteins which bind phosphorylcholine (*2*). For convenience, the myeloma-specific determinants have been referred to as "idiotypes" and the antiserums as "anti-idiotypic serums" (*3*). To test the biological activity of the anti-idiotypic serums, we devised the following methods: (i) an antibody response specific for phosphorylcholine was regularly induced by immunizing BALB/c mice or cultures of spleen cells with heat-killed pneumococci; and (ii) the plaque-forming cell (PFC) technique was modified to demonstrate single cells synthesizing antibody to phosphorylcholine. We find that the anti-idiotypic serums inhibit formation of plaques; apparently the idiotypic determinants in myeloma proteins which bind phosphorylcholine and those in antibodies produced in a primary response to pneumococci are identical or very similar.

Purified monomeric IgA myeloma proteins obtained from BALB/c mice carrying the TEPC-15 or the HOPC-8 tumors were donated by Dr. M. Potter. Antiserums against these myeloma proteins which bind phosphorylcholine were prepared in A/He mice (*2*). The antiserums were absorbed with normal BALB/c serum; they reacted only with those myelomas which bind phosphorylcholine and share specific antigenic determinants (*2*).

Table 1. Primary immune response to pneumococcus in vivo. Inhibition of plaques by phosphorylcholine and anti-idiotypic serums. BALB/c mice were immunized with 10^9 heat-killed pneumococci, strain R36A. Spleens from at least three mice were assayed for PFC at day 4 against SRBC, PnC-SRBC, and PC-SRBC. Prior to addition of guinea pig complement, the slides were incubated with either A/He normal mouse serum (NMS), phosphorylcholine, or one of the anti-idiotypic serums (HOPC-8, TEPC-15). The number of plaques is given per 10^7 spleen cells and represents the average of at least four slides.

Target cell	Inhibition of PFC						
	NMS 10^{-3}	Phosphorylcholine		Anti-HOPC-8		Anti-TEPC-15	
		$10^{-3}M$	$10^{-6}M$	10^{-3}	10^{-6}	10^{-3}	10^{-6}
	Heat-killed rough pneumococci, strain R36A						
SRBC	10						
PnC-SRBC	4816	480	6840	100	4520	60	5160
PC-SRBC	5120	40	6680	160	5760	64	5480
	SRBC						
SRBC	2160	1864		1972		2132	

A primary PFC response to phosphorylcholine was obtained by injecting BALB/c female mice intravenously with 10^9 heat-killed rough pneumococci, strain R36A. Spleens were removed after 4 days, and PFC were enumerated by means of the slide modification of the Jerne-Nordin hemolytic plaque technique (*4*). Spleen cell suspensions were assayed against either sheep red blood cells (SRBC), SRBC coated (*5*) with pneumococcal C polysaccharide (PnC-SRBC), or SRBC conjugated (*6*) with phosphorylcholine (PC-SRBC). Slides were incubated for 2 hours in either (i) a 10^{-3} dilution of normal A/He serum, (ii) 10^{-3} or $10^{-6}M$ phosphorylcholine, or (iii) 10^{-3} or 10^{-6} dilutions of either anti-idiotypic serum. The slides were washed in saline for 15 minutes before incubation with a 1 : 25 dilution of guinea pig complement (GPC) for 1 hour. As shown in Table 1, spleen cells obtained from mice immunized with pneumococci give a very low background response when assayed against SRBC as target cells. The PFC response was high when the spleen cells were assayed against either PnC-SRBC or PC-SRBC in the presence of normal A/He serum. Formation of plaques was completely inhibited when the spleen cells were incubated with $10^{-3}M$ phosphorylcholine or with either idiotypic antiserum diluted 1 : 1000; at higher dilutions no inhibition of plaques could be detected (*7*). Table 1 also shows that neither phosphorylcholine nor the anti-idiotypic serums inhibited plaque formation by spleen cells from mice immunized with SRBC (*8*).

The method of Mishell and Dutton (*9*) was modified to obtain a primary response to phosphorylcholine in vitro (*10*). Cultures containing a suspension of 10^7 spleen cells obtained from normal BALB/c female mice were immunized with 10^6 heat-killed rough pneumococci, strain R36A; each culture received daily 0.05 ml of a nutritional mixture (*9*) and 0.05 ml of fetal calf serum. At day 4, the cells in culture were dispersed and assayed for PFC against SRBC, PnC-SRBC, or PC-SRBC. Slides were incubated for 2 hours in normal A/He serum, phosphorylcholine, or anti-idiotypic serums, guinea pig complement was added, and the slides were incubated further for 1 hour (Table 2). The number of plaques obtained in the presence of normal A/He serum with either PnC-SRBC or PC-SRBC as target cells was high, while the background to SRBC was low. High concentrations of phosphorylcholine or of anti-idiotypic serum completely inhibited plaque formation against PnC-SRBC or PC-SRBC; no inhibition occurred with the lower concentrations. As controls, some spleen cell cultures were immunized with SRBC, and the PFC response was measured at day 4 (Table 2). No inhibition of plaque formation was observed when the slides were incubated with either phosphorylcholine or anti-idiotypic serums.

In separate experiments, we have found that the rate of appearance and the magnitude of the PFC response to phosphorylcholine in mice is comparable to the response obtained in spleen cell cultures. Furthermore, formation of plaques by spleen cells after primary immunization of mice or cultures is equally susceptible to inhibition by phosphorylcholine or anti-idiotypic serums (*11*). This inhibition is antigen specific since plaque formation to an unrelated antigen (SRBC) is not affected by either of the inhibitors.

Pneumococcal polysaccharides are generally considered weak immunogens since they elicit poor primary antibody responses (*12*). The pneumococcal C polysaccharides are, however, excellent antigenic determinants in that large amounts of polysaccharide–specific antibodies are produced in several species immunized with the whole pneumococcal organism (*13*). The observed PFC response against either the pneumococcal C polysaccharide or phosphorylcholine are almost identical (Tables 1 and 2). Furthermore, phosphorylcholine inhibits equally plaque formation when either PnC-SRBC or PC-SRBC are used as target cells. Choline is linked to a polysaccharide moiety of the R36A pneumococcal cell wall (*14*); possibly, the simple chemical structure of phosphorylcholine mimics a predominant

Table 2. Primary immune response to pneumococcus in vitro. Inhibition of plaques by phosphorylcholine and anti-idiotypic serums. Spleen cells from normal BALB/c mice were immunized in vitro with 10^6 heat-killed pneumococci, strain R36A. Cell cultures were disrupted at day 4 and assayed for PFC against SRBC, PnC-SRBC, and PC-SRBC. Prior to additions of guinea pig complement, the slides were incubated with either A/He normal mouse serum (NMS), phosphorylcholine, or one of the anti-idiotypic serums (HOPC-8, TEPC-15), diluted as indicated. The number of plaques is given per 10^7 spleen cells per culture and represents the average of counts on six slides; the slides were duplicates from each of triplicate cultures.

Target cell	Inhibition of PFC (10^7 spleen cells per culture)						
	NMS 10^{-3}	Phosphorylcholine		Anti-HOPC-8		Anti-TEPC-15	
		$10^{-3}M$	$10^{-6}M$	10^{-3}	10^{-6}	10^{-3}	10^{-6}
	Heat-killed rough pneumococci, strain R36A						
SRBC	326						
PnC-SRBC	2470	239	3172	83	2704	78	2584
PC-SRBC	2444	312	2028	343	2395	268	2080
	SRBC						
SRBC	2861	3108		3396		3149	

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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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