body, and it is not certain whether an extract or an antigen preparation will necessarily be equally useful for measuring both types of response. That is something that needs to be established and may even vary with the antigen source.

It may be that more than one type of extract will be required for each source, perhaps one that would be suitable for precipitin testing, one for IgE testing, or some other kind of testing such as cellular responses. As soon as a reasonable scientific base develops in terms of relating antigen preparations to measurement of disease, one can develop suitable reference preparations. If human serum is required as a reference preparation, (fortunately plasmapheresis makes this readily available in large quantities) it can be freeze-dried and kept fairly stable. Animal sera, useful for measuring precipitating antigens is even easier to obtain since animals are relatively easy to immunize; consequently, I think a program can be started in limited areas if one develops the appropriate scientific base.

Involvement of the complement system in hypersensitivity pneumonitis

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It has been said that the complement system is the principal humoral affecter mechanism responsible for elimination of foreign material from a host. Although this may be an overstatement, there is little doubt that complement mediation of a number of reactions plays an important role in host defense. The role of the complement system in certain types of hypersensitivity reactions has been well documented. The role of the complement system in hypersensitivity pneumonitis (HP) however has until recently not received a great deal of attention.

A few years ago, Dr. Berrens reported his observations on the ability of antigens associated with pigeon excreta to consume hemolytic complement activity. He described the presence of an unusually labile complement component demonstrable in the serum of patients with pigeon breeder’s lung when exposed in vitro to these antigens. The consumption of complement could not be attributed to the presence of hydrolytic enzymes also present in this material. Although they were not able to eliminate the possibility of low levels of immunoglobulin, their results strongly suggested an activation of the alternative pathway of complement.

In conjunction with Dr. V. Moore, we have been able to confirm the suspicions of Dr. Berrens with a fraction of pigeon dropping extract (PDE), and in addition have shown that a number of the early components of the classical pathways also seem to be affected. More recently Drs. Edwards and Flaherty and I demonstrated activation of the alternative pathway induced by a number of antigens associated with HP, particularly farmer’s lung disease (FLD).

Although there has been some discrepancy between ourselves as to the nature of the antigens responsible, it does appear that the generation of active complement components could present answers to questions related to the mechanism of pathogenesis.

Although far from complete, I would like to present some observations we have made on the interaction of extracts of the thermophilic actinomycetes associated with HP and the complement system, as well as to present some preliminary experiments attempting to isolate the responsible antigens.

In a very simplistic view of the complement cascade, one can distinguish 3 distinct sections: The first section is reactions leading to a generation of C3 convertase; the second portion results in the activation of the third component; and the last portion is the activation of the terminal components of the cascade. Antigens reacting with antibody, particularly of the IgG and IgM classes and with respect to IgG, particularly the first and third subclass, react with the first component of complement which in turn activates C4 and C2 to the production of a C3 convertase. This reaction requires calcium and magnesium as cofactors and that is important in attempting to understand the procedures we are currently using.

Alternatively C3 convertase can be generated nonimmunologically by a factor derived from cobra
venom, inulin, endotoxin, zymosan, or aggregates of both subclasses of IgA and possibly aggregates of the fourth subclass of IgG. There is some question as to whether the immunoglobulins in conjunction with antigen can activate the system. However, I would like to emphasize the conversion of C3 proactivator or properdin factor B to its activated form, C3 activator, which in turn generates active C3. In addition there is a loop initiated by C3b in the presence of factor B and C3Phase (factor D) which generates more C3b. This is referred to as the amplification loop.

The techniques we are using to define alternate and classical pathway activity utilize the differential chelation of calcium and magnesium. By the selective chelation of calcium by ethyleneglycoltetracetic acid (EGTA), we can measure alternative pathway activity. We have also used the conversion of C3 proactivator (factor B) to the activated form (factor B) as defined immunoelectrophoretically as a means of assessing alternative pathway activity.

In our laboratory, using two classical activators of the alternate pathway, we have utilized a pool of fresh human serum as a source of complement. The pool we are using was obtained from normal healthy individuals previously screened for immunoglobulins to the antigens that we are working with by immunodiffusion, complement fixation, and passive hemagglutination. When the pool is incubated with zymosan or inulin in the absence of any chelator, we see a dose-dependent decrease in the amount of complement remaining in that serum. We can inhibit this reaction almost completely by ethylenediaminetetraacetic acid (EDTA) in both instances. However, as we do this same experiment chelating the serum with EGTA and excess magnesium in place of EDTA, complement consumption is very similar to that in the unchelated serum. We can compare degree of consumption by comparing the slope of the linear regression.

As the alternate technique, an immunoelectrophoretic conversion of C3PA (factor B) is induced by a substance capable of activating the alternative pathway; this should be inulin and not insulin. Normal human serum alone in this system gives a single arc in the B region. After incubation with one of these reagents this arc is converted or is split to gamma or more cathodal migrating protein, suggestive that the reaction is proceeding through the alternative pathway. This represents our first attempts at associating the system with the thermophilic actinomycetes in that an extract of *M. faeni* is capable of converting C3PA to the activated form. It should be noted that this reaction also proceeds in the presence of EGTA but not EDTA. The results of the dose relationship to two different batches of *M. faeni* with normal human serum are presented as the percent complement consumption in the presence of these two extracts. Again there is a dose related consumption of whole hemolytic complement activity.

Dr. Roberts will later discuss these extracts in relation to the presence of proteolytic enzyme activity present. We wanted to look at the effect of these proteolytic enzymes on the complement system and whether they were the agents responsible for this complement activation. In the system that he will describe diisopropyl fluorophosphate will completely inhibit the proteolytic activity with *M. faeni* and *T. vulgaris*. Our results show the percent complement consumption on the DFP-treated extracts and there is basically no difference between the ability of *M. faeni* and *T. vulgaris* to activate complement after treatment with diisopropyl fluorophosphate.

Attempts to separate or to isolate the antigens or the agents responsible for this complement activation were represented by an elution pattern of *M. faeni* on ultragel 54, a method of gel filtration with the use of a 5% acrylamide, 4% agarose gel. We see at least three demonstrable peaks that have been labeled UG1, UGII, UGIII. We appear to be, at least with the first two fractions, separating out a number of proteins in these extracts. When we look at the effect of each of these fractions on the complement system, we see a large difference between this first fraction and the second two.

The complement consumption by the whole extract in the presence and absence of a chelator was measured. The complement consumption is not completely inhibited by EDTA, and to a lesser extent by EGTA. When we look at the first fraction set up at the same concentration as the original extract we see a greater than 90% reduction of the hemolytic complement activity, and again not completely inhibited by either of these chelators. There seems to be very little activity associated with either of the other two fractions and I am assuming, for the present time, that the small amount of activity in UGIII is due to the overlap between UG1 and UGII. The immunoelectrophoretic conversion of C3PA is inducible by the crude extract and by the first fraction. The second fraction also induces the C3PA, and, as with the previous results, I do not know whether this is an overlap between these two fractions.

We attempted to resolve or to confirm this consumption by measuring the early components of the classical pathway. If this activity that we are seeing is proceeding exclusively through the alternate pathway, we should see a depletion in the third component of complement with little or no effect on the fourth component. However, the results we found were not at all
along that order. Both the crude extract and UG1 induce a significant decrease in the amount of C3, but, surprisingly to us, the C4 was reduced even more. We have gone on and made a few attempts to measure the first component of complement to see whether or not this activity was directly affecting C4 and entering the cascade at this point or whether it was acting as an immune complex and binding C1 and in that way initiating the pathway. Our first attempts have suggested that the C1 hemolytic titer is not affected by either of these extracts. Consequently, we feel that what we are seeing is an initiation of the complement sequence through the fourth component.

When we started these experiments we observed what we thought was an anomaly. The *M. faeni* extract, an extract of *T. vulgaris*, and an extract of what Dr. Kurup has called *T. candidus* seem to have no effect on guinea pig complement, whereas they were very potent inactivators of human complement system (unlike inulin which has a similar effect on both human and guinea pig serum). This is indeed true if we inject each one of these substances in vivo. There is significantly less consumption of complement induced by *M. faeni* than there is by inulin over a 24-hr period. At the time we thought that this was rather unique, but after going back through the literature more thoroughly I realized that Dr. Berrens reported this with pigeon breeders in his original article. Since then he has also talked about this same phenomenon with a number of antigens associated with type I allergies.

In summary, I have attempted to present some of the observations we have made on the interaction of an extract of *M. faeni* with the complement system. It is apparent that antigens associated with this organism, as well as other thermophilic actinomycetes, can consume complement. The mechanism of this complement consumption is not entirely understood but does involve components of both the classical and the alternate pathways.

However, it is not clear as to whether the same antigens are responsible for activation of both pathways. It is possible that levels of antibody below detectable limits by the methods that we employed are responsible for the C4 consumption, whereas other antigens are associated with the conversion of the alternate pathway components. It is also conceivable that conversion of C3PA is a function of the amplification loop, that is, the feedback of C3b on the C3 proactivator and in that way generating this activated form of the enzyme.

A number of reports have demonstrated that proteolytic enzymes are capable of generating active C3. These enzymes are serine proteases and act on C3 directly. In the present study, DFP, a potent inhibitor of serine-histidine proteases, has no effect on the ability of the extracts to consume complement but effectively inhibited the caseinolytic activity that Dr. Roberts will discuss.

Such a mechanism would not explain the decrease in C4 observed with both the whole extract and with the first fraction. Consequently, we do not feel that the consumption of complement is due to these proteolytic enzymes.

With respect to the involvement of this interaction with the pathogenesis we cannot make any conclusions. However, this mechanism certainly presents an attractive proposal for those patients with obvious clinical disease in which antibody has not developed or we cannot identify it.

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**Humoral and cellular immunologic aspects of hypersensitivity pneumonitis**

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The predominant concept concerning the pathogenic mechanisms of hypersensitivity pneumonitis (HP) is that the disease is caused by immune complexes. However, recent studies in patients and in animal models have indicated that cellular hypersensitivity may also play a role in the pathogenesis of this group of diseases. My discussion will involve studies that have been performed on the humoral antibody (Ab) and cellular immunologic response of personnel, as well as experimental animals, which have been exposed to some of the etiologic agents of HP.

I will concentrate on pigeon breeder’s disease.