cubation at 37°C for 3 hr with diisopropyl fluorophosphate (DIFP), p-tosyl-L-lysine chloromethyl ketone (TLCK), and phenylmethylsulfonyl fluoride (PMSF) over a concentration range of 10^{-2} to 10^{-3} M. The most definitive data in this regard was obtained with (p-amidinophenyl) methanesulfonyl fluoride (pAPMSF), an active site titrant that irreversibly inactivates serine proteases which demonstrate substrate specificity for the positively charged side chains of the amino acids lysine or arginine by sulfonation of the active site serine (Laura, *et al.*, Biochem. *19*:4859, 1980). pAPMSF completely inhibited C6 functional activity at a concentration of 10^{-4} M. The synthetic substrate acetylglycyl-L-lysine methyl ester was able to fully protect C6 from inactivation by pAPMSF, DIFP, or PMSF, but was unable to protect C6 from inactivation by $HgCl_2$ or pCMB. These results strongly suggest C6 is a serineprotease and C6 esterolytic activity is essential for expression of C membrane attack complex membranolytic function. (Supported by Grant CA-24447, NCI).

DIFFERENTIAL EFFECTS OF C5a AND F MET-LEU-PHE ON THE PRODUCTION OF O_2^- AND PROSTAGLANDIN BY MACROPHAGES *Steven L. Kunkel*, Michael C. Plewa, Joseph C. Fantone and Peter A. Ward.* Dept of Pathology, Univ of Michigan Med School, Ann Arbor, Mi.

Stimulated mononuclear phagocytes synthesize and release a plethora of inflammatory mediators. This is often dependent both on the inflammatory stimulus as well as the source of the mononuclear phagocytes. We have found this to be especially true concerning the ability of two potent chemotactic stimuli, C5a and N-formylmethionylleucylphenylalanine (FMLP), to release prostaglandins and superoxide anion (O_2^-) from mouse peritoneal macrophage populations. Over a dose range of 10^{-5} to 10^{-9}M, FMLP induced the release of low levels of O_2^- (2.4 to 6.8 nmoles/10^6cells/2 hr) from thioglycollate elicited macrophage. Resident macrophages stimulated with the above concentrations of FMLP produced no significant increase in O_2^- levels. C5a, at does ranging from 1 ug/ml to 10 ug/ml, induced the release of 3.7 to 7.5 nmoles $O_2^-/10^6$ cells/2 hr. from elicited macrophages. As with FMLP, C5a produced no significant increase in O_2^- levels from resident macrophages. Although FMLP was not effective in stimulating the release of protaglandins from either macrophage populations, C5a significantly stimulated the release of prostaglandin E_2 (PGE_2) from both elicited and resident macrophages. Resident macrophages released up to 40 times the basal level of immunoreactive prostaglandin E_2 (PGE_2) when exposed to 5 ug/ml C5a ($4x10^{-7}$M) over a 2 hour period, whereas, thioglycollate elicited macrophage released only a 2-3 fold increase in basal PGE_2 levels. Upon stimulation with C5a, thromboxane levels from resident or elicited macrophages were elevated approximately 3 fold. These data suggest that there may be a fundamental difference between various macrophage cell populations, inflammatory stimuli, and subsequent mediator release in a given inflammatory reaction.

THE BREAKDOWN OF C3bi TO C3c, C3d and C3g. *P.J. Lachmann**, M I T I, MRC Centre, Cambridge, England and *M.K. Pangburn.* Scripps Clinic, La Jolla, California, USA.

C3bi - the end product of short term C3 activation - is further broken down and this process has been studied using the monoclonal anti-C3 antibodies described by Lachmann et al (1980) Immunology *41*: 503.

It has been shown that:

1. Both on surfaces and in solution the initial cleavage of C3bi (M Wt 186K) is into fragments, C3c (M Wt 143K) and α 2d globulin (M Wt 43K). α 2d globulin is of fast mobility and carries the determinants for two anti-C3 Mc Abs - clone 3 (anti-C3d) and clone 9 (now shown to be anti-C3g).
2. α 2d is further cleaved into C3d (M Wt 35K) and C3g (M Wt 8K). C3d being left attached to surfaces. In solution C3d is of slower electrophoretic mobility than C3c. It reacts with clone 3. C3g cannot be precipitated by anti-C3 antisera but reacts with clone 9.
3. Plasmin and low concentrations of thermolysin and trypsin (c 100 mg/ml) split C3bi to C3c and C3d,g. Higher concentrations cause breakdown to C3d and C3g. Plasmin is the most likely enzyme to bring about the initial split *in vivo* but serum depleted of plasminogen still supports the split and streptokinase does not accelerate it. The cleavage of C3d,g to C3d and C3g may not occur physiologically at least in the circulation. It occurs in serum *in vitro* only in the absence of sodium azide and red cells coated with C3 *in vivo* in cold agglutinin disease are coated with C3d,g and not with C3d.

The fragment here called C3g was previously called C3e following the usage of Harrison and Lachmann (1980) Molecular Immunology 17:219. Since it is likely that it is distinct from the C3e described by Ghebrehiwet and Müller-Eberhard (1979) J. Immunol., 123:616, the designation has been changed to C3g.

NICOTINIC CHOLINERGIC RECEPTORS ON HUMAN AND GUINEA PIG MONONUCLEAR PHAGOCYTES MODULATE C2 SYNTHESIS. *D. Lappin*, T. Barkas* and *K. Whaley.* Pathology Department, Western Infirmary, Glasgow and Southern General Hospital, Glasgow, Scotland.

When acetylcholine (ACH) or carbachol are added to cultures of human monocytes or guinea pig macrophages (resting or elicited), enhancement of C2 synthesis occurs. Pilocarpine is ineffective. The enhancement produced by acetylcholine is abrogated by D-tubocurarine or Bungarotoxin, but not by atropine. Thus the receptor through which these effects are mediated, is a nicotinic cholinergic receptor.

IgG (and its $F(ab')_2$ fragment) prepared from sera of patients with myasthenia gravis who had high levels of antibody of skeletal muscle ACH receptors, inhibited the effect of ACH as did IgG and $F(ab')_2$ prepared from an antiserum to the ACH receptor of the electroplaques of Torpedo marmorata. Thus the mononuclear phagocyte ACH receptor is antigenically similar to the receptors of human skeletal muscle and the electroplaques of Torpedo marmorata. The biological significance of the receptor is currently unknown.