Immunology 1978 **35** 369

Production of C2 by human alveolar macrophages

S. K. ACKERMAN, P. S. FRIEND, J. R. HOIDAL & S. D. DOUGLAS *Department of Medicine, University of Minnesota Medical School, Minneapolis, Minnesota* 55455, *U.S.A.*

Received 17 *November* 1977; *accepted for publication* 21 *December* 1977

Summary. Human and rat alveolar macrophages produce haemolytic C2 during *in vitro* culture. We conclude that C2 synthetic ability is maintained during *in vivo* maturation of human monocytes to macrophages and that production of complement components by mature tissue macrophages may be important for optimal generation of inflammatory responses.

INTRODUCTION

Mononuclear phagocytes, in addition to their roles in phagocytosis and immune regulation, also function as secretory cells, and release a variety of specific humoral products of potential importance to the regulation of inflammation. These products include lysozyme (Osserman & Lawlor, 1966), lysosomal enzymes (Davies, Allison & Haswell, 1973; Weissman, Dukor & Zurier, 1971), plasminogen activators (Unkeless, Gordon & Reich, 1974), collagenase (Wahl, Wahl, Mergenhagen & Martin, 1974), and early complement components (Bentley, Bitter-Suerman, Hadding & Brode, 1976; Colten, 1972a, 1972b; Ilgen, Bossen, Rowlands & Burkholder, 1974; Stecher & Thorbecke, 1967). For example, synthesis of functionally inactive C3 and C4 has been demonstrated during *in vivo* culture of both peripheral blood monocytes (Einstein, Schneeberger & Colten, 1976) and human peritoneal macrophages (Stecher, Morse & Thorbecke, 1967). In addition, human peripheral blood monocytes synthesize haemolytically active C2 *in vitro*; after an initial lag period, monocytes secrete C2 at a rate which increases with duration of culture (Einstein *et al.*, 1976). Also, lymphokine-rich supernatants prepared from antigen-stimulated lymphocytes appear to enhance C2 production (Littman & Ruddy, 1977). Whether C2 production accompanies *in vivo* differentiation of human monocytes to macrophages, however, is not known. Since the more mature tissue macrophage has a major role in inflammatory processes, it is necessary to study this cell when evaluating the relationship between secreted products and inflammation. We have, therefore, studied human and rat pulmonary macrophages and show here that both cells produce haemolytically active C2.

Correspondence: Dr Steven D. Douglas, University of Minnesota Medical School, Mayo Box 1, Minneapolis, MN 55455, U.S.A.

0019-2805/78/0800-0369$02.00

MATERIALS AND METHODS

Human alveolar macrophages were obtained from adult male smokers by transnasal fibreoptic bronchoscopy with pulmonary lavage (Daughaday & Douglas, 1976); rat alveolar macrophages were obtained by saline lavage of lungs from adult Sprague-Dawley rats killed with Nembutal. Washed human or rat cells were plated on 15 mm glass

coverslips (Bellco, Vineland, N.J.) in the bottom of Linbro plastic culture dishes (Linbro, New Haven, CT) in 2 ml of RPMI 1640 containing 10% heat-inactivated (56°, 30 min) foetal calf serum, 2 mM glutamine and antibiotics ('complete medium'). From 10^5 to 10^6 cells were added into each well and cultured overnight at 37° in a 5% CO_2 atmosphere. Coverslips were then gently rinsed clear of non-adherent cells and 2 ml of fresh complete medium were added. Human alveolar macrophages prepared in this manner were a morphologically uniform population of cells which contained phagocytosed debris. Ninety to 95% of cells expressed both Fc and C3 receptors as determined by rosette formation with 7S EA and 19S EAC, respectively (Douglas & Huber, 1972). Rat macrophages were highly vacuolated but lacked interiorized debris; approximately 90% of these cells rosetted with 7S EA. After the coverslips were rinsed, incubation at 37° was resumed. At 1 to 10 day intervals 200 μl aliquots of supernatant were removed for determination of C2 activity with replacement into the well of 200 μl of fresh complete medium. Haemolytic C2 was measured by a sensitive functional assay previously described (Repine, Clawson & Friend, 1977) utilizing rat EDTA-serum as the source of terminal complement components. Fresh complete medium was routinely assayed along with experimental samples and showed no detectable C2 activity.

RESULTS

Figure 1 shows the results of a representative experiment for human alveolar macrophages. Haemolytic C2 was detectable in the supernatant by day 1 (total duration of culture, 36 h) and increased linearly with time; thus, there was no apparent lag

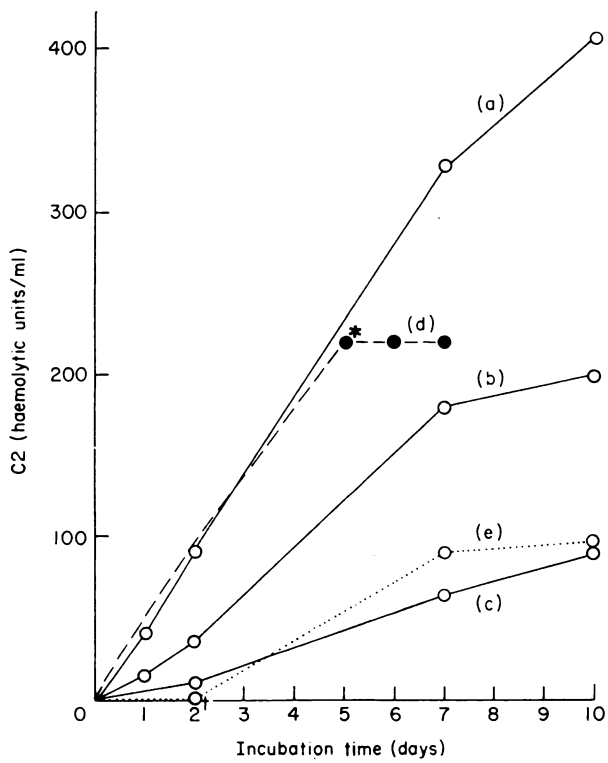

Figure 1. C2 synthesis by human alveolar macrophages: (a) 10^6 cells, (b) 5×10^5 cells, or (c) $2{\cdot}5 \times 10^5$ cells added to wells and treated as described in text. (d) 10^6 cells added to well, cultured for 5 days, then cycloheximide added at final concentration of 1·5 μg/ml. (e) 10^6 cells added to well with cycloheximide 1·5 μg/ml added after rinsing of nonadherent cells. Forty-eight h later, cells were rinsed and 2 ml of complete medium without cycloheximide were added and incubation was continued. During the initial 48 h incubation with cycloheximide many cells died and detached from coverslips, which is reflected in decreased rate of synthesis in curve (e) compared to (a). All samples were kept at −70° until assayed and values shown are corrected for dilution.

period. C2 production was reversibly inhibited by cycloheximide (1·5 μg/ml) and was directly related to the number of cells originally added to the wells. Similar results were obtained with rat alveolar macrophages, thus establishing the phenomenon for a second species. We tested the stability of the synthesized C2 in our system in two ways. Supernatant removed from human macrophage cultures lost no C2 activity during 5 days of additional cell-free incubation at 37°. Furthermore, supernatants from cultures to which cycloheximide was added after 5 days of C2 production also showed no decrease in activity during an additional 48 h incubation (Fig. 1). We concluded that in our system there was no detectable degradation of C2 either by secreted macrophage products or by the cells in the absence of protein synthesis.

DISCUSSION

We believe these findings are significant in several respects. As indicated, they demonstrate that the capacity for C2 synthesis is retained during *in vivo* differentiation of human monocytes to macrophages. Although because of different assay systems we could not directly compare the rate of synthesis of alveolar macrophages with that reported for peripheral blood monocytes (Einstein *et al.*, 1976), the absence of a lag period in our system suggests an enhanced capacity for C2 production in the macrophage. Secondly, the production of C2 by alveolar macrophages may be important in the generation of local inflammatory responses. Complement components other than C2 have been found in the respiratory tract. Haemolytic C4 and C6 are detectable in bronchoalveolar lavage fluid from healthy smokers and non-smokers (Reynolds & Newball, 1974) and in lavage fluid from patients with interstitial pulmonary fibrosis and chronic hypersensitivity pneumonitis (Reynolds, Fulmber, Kazmierowski, Roberts, Frank & Crystal, 1977). Also, a chemotactically active peptide, tentatively identified as C5a, has been found in lavage fluid from normal rhesus monkeys (Kazmierowski, Gallin & Reynolds, 1977).

The presence of multiple components suggests a functional role for complement in the respiratory tract. Several specific interactions between complement components and inflammatory cells have recently been described which may be related to the maintenance of a sterile respiratory tract and to the generation of an adequate inflammatory response during infection. It has been shown that C3b can selectively release macrophage lysosomal enzymes and that macrophage proteinases can in turn cleave C3b (Schorlemmer & Allison, 1976); this sequence could lead to local amplification of complement activation. Also, macrophage enzymes can generate chemotactically active C5a from C5 (Snyderman & Dannenberg, 1972) and primate macrophages cultured *in vitro* produce a distinct factor chemotactic for neutrophils (Kazmierowski *et al.*, 1977). In addition, neutrophil lysosomal enzymes can activate factor B of the alternative pathway (Goldstein & Weissman, 1974). Finally, as we have recently shown (Repine *et al.*, 1977), C2 is required for optimal neutrophil killing of *Staphylococcus aureus*. Thus numerous possibilities exist for complex interactions between macrophages, neutrophils and complement during inflammation. Secretion of complement components by inflammatory cells may be an important link in these interactions. In particular, the alveolar macrophage, which is the first line of defence against inhaled pathogens, may play a vital role in the rapid mobilization of host defence mechanisms.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health, USPHS No. AI 12478 and NIH postdoctoral fellowship for Dr Ackerman, No. 1F 32 AI 05447, and by grants from the Kroc Foundation, the National Leukemia Association, and the General Mills Foundation. Dr. Friend is the recipient of an NIAMMD Clinical Investigation award, AM00445.

REFERENCES

BENTLEY C., BITTER-SUERMAN D., HADDING U. & BRODE E. (1976) *In vitro* synthesis of factor B of the alternative pathway of complement activation by mouse peritoneal macrophages. *Eur. J. Immunol.* **6**, 393.

COLTEN H.R. (1972) *In vitro* synthesis of a regulator of mammalian gene expression. *Proc. nat. Acad. Sci.* **69**, 2233.

COLTEN H.R. (1972) Ontogeny of the human complement system: *in vitro* biosynthesis of individual complement components by fetal tissues. *J. clin. Invest.* **51**, 725.

DAUGHADAY C.C. & DOUGLAS S.D. (1976) Membrane

receptors on rabbit and human pulmonary alveolar macrophages. *J. Reticuloendothel. Soc.* **19,** 37.

DAVIES P., ALLISON A.C. & HASWELL A.D. (1973) Selective release of lysosomal hydrolases from phagocytic cells by cytochalasin B. *Biochem. J.* **134,** 33.

DOUGLAS S.D. & HUBER H. (1972) Electron microscopic studies of human monocyte and lymphocyte interaction with immunoglobulin and complement-coated erythrocytes. *Exp. Cell Res.* **70,** 161.

EINSTEIN P., SCHNEEBERGER E.E. & COLTEN H.R. (1976) Synthesis of the second component of complement by long-term primary cultures of human monocytes. *J. exp. Med.* **143,** 114.

GOLDSTEIN I.M. & WEISSMAN G. (1974) Generation of C5-derived lysosomal enzyme-releasing activity (C5a) by lysates of leukocyte lysosomes. *J. Immunol.* **113,** 1583.

ILGEN C.L., BOSSEN E.H., ROWLANDS D.T. & BURKHOLDER P.M. (1974) Isolation and characterization of C4-synthesizing cells from guinea-pig spleen. *Immunology,* **26,** 659.

KAZMIEROWSKI J.A., GALLIN J.I. & REYNOLDS H.Y. (1977) Mechanism for the inflammatory response in primate lungs. Demonstration and partial characterization of an alveolar macrophage-derived chemotactic factor with preferential activity for polymorphonuclear leukocytes. *J. clin. Invest.* **59,** 273.

LITTMAN B.H. & RUDDY S. (1977) Production of the second component of complement by human monocytes: stimulation by antigen-activated lymphocytes or lymphokines. *J. exp. Med.* **145,** 1344.

OSSERMAN E.F. & LAWLOR D.P. (1966) Serum and urinary lysozyme (muraminidase) in monocytic and monomyelocytic leukemia. *J. exp. Med.* **124,** 921.

REPINE J.E., CLAWSON C.C. & FRIEND P.S. (1977) Influence of a deficiency of the second component of complement on the bactericidal activity of neutrophils *in vitro*. *J. clin Invest.* **59,** 802.

REYNOLDS H.Y. & NEWBALL H.H. (1974) Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. *J. lab. clin. Med.* **84,** 559.

REYNOLDS H.Y., FULMBER J.D., KAZMIEROWSKI J.A., ROBERTS W.C., FRANK M.M. & CRYSTAL R.G. (1977) Analysis of cellular and protein content of bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis. *J. clin. Invest.* **59,** 165.

SCHORLEMMER H.U. & ALLISON A.C. (1976) Effects of activated complement components on enzyme secretion by macrophages. *Immunology,* **31,** 781.

SNYDERMAN R., SHIN H.S. & DANNENBERG A.M. (1972) Macrophage proteinase and inflammation: The production of chemotactic activity from the fifth component of complement by macrophage proteinase. *J. Immunol.* **109,** 896.

STECHER V.J. & THORBECKE G.J. (1967) Sites of synthesis of serum proteins. I. Serum proteins produced by macrophages *in vitro*. *J. Immunol.* **99,** 643.

STECHER V.J., MORSE J.H. & THORBECKE G.J. (1967) Sites of production of primate serum proteins associated with the complement systems. *Proc. Soc. exp. Biol. Med.* **124,** 443.

UNKELESS J.C., GORDON S. & REICH E. (1974) Secretion of plasminogen activator by stimulated macrophages. *J. exp. Med.* **139,** 834.

WAHL L.M., WAHL S.M., MERGENHAGEN S.E. & MARTIN G.R. (1974) Collagenase production by endotoxin-activated macrophages. *Proc. nat. Acad. Sci.* **71,** 3598.

WEISSMAN G., DUKOR P. & ZURIER R.E. (1971) Effect of cAMP on release of lysosomal enzymes from phagocytes. *Nature (New Biol.),* **231,** 131.