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Production of functional complement by human mononuclear phagocytes

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Complement is probably the most ancient known humoral defense system against pathological agents. Receptors for human factor C3 has been demonstrated on echinoid phagocytes from the sea urchin that belongs to the invertebrates [3]. Moreover, lytic complement like activity has been detected in the sea urchin coelomic fluid, as demonstrated by lysis of co-incubated rabbit erythrocytes. The complement system which consists of about 20 factors is required for onset of the inflammatory response, which is a prerequisite for host defense to infections.

A key question is whether mononuclear phagocytes in situ in the tissues have the potential to produce the complete functional complement system with generation of complement induced activites including opsonization, chemotaxis, anaphylaxis and lysis of microorganisms.

Identification of phagocyte produced complement

The story on complement production in mononuclear phagocytes started in 1967 [17], when synthesis of C3 and C4 by peritoneal macrophages was shown. The methods were based on detection of soluble components in the cell culture medium [12] by functional hemolytic assays or by radioimmunoassay (RIA) where the factors are identified by their antigenic properties without any functional analysis. The component could also be identified by autoradiography combined with a RIA of the medium from cell-cultures incubated with radioactive amino acids. In addition, sodium dodechyl sulphate (SDS) electrophoresis can be used to determine the molecular weight of the component focused upon.

Using these methods, the complement components depicted in Table I are known to be synthesized by [12] human monocytes/macrophages.

However, it still remained to find out whether the terminal components C6-C9 were synthesized, and to ascertain whether the phagocytes produced complement as a functional entity – namely the functional alternative-, classical- and possibly terminal (C5-C9) pathway.

In this work we incubated [8, 9, 10, 11, 16] activators of the classical-

TABLE I

Synthesis of some complement components by human mononuclear phagocytes

Component	Source	Year (reference)
C3	Blood	—75 (13), —83 (19)*
	Peritoneum	—67 (17)
	Lung	—83 (4)
	Breast milk	—82 (5)
I	Blood	—80 (20)
	Synovial fluid, RA	—80 (6)*
Clq/Cl	Blood/peritoneum	—78 (15)/—67 (18)
ClINH	Blood	—83 (2), —85 (14)*
C2	Blood	—76 (7)*
	Lung	—78 (1)*
	Breast milk	—82 (5)*
	Synovial fluid, RA	—80 (6)*
C4	Blood	—80 (20)
	Peritoneum	—67 (17)
	Synovial fluid, RA	—80 (6)*
B	Blood	—80 (20)*
	Lung	—83 (4)*
	Breast milk	—82 (5)*
	Synovial fluid, RA	—80 (6)*
D	Blood	—80 (20)*
	Synovial fluid, RA	—80 (6)*
H	Blood	—80 (20)*
	Synovial fluid, RA	—80 (6)*
P	Blood	—80 (20)*
	Synovial fluid, RA	—80 (6)*
C5	Blood	—80 (20)

Abbreviation: Rheumatoid arthritis (RA)
* Point at which component was functionally active

(lgM sensitized sheep erythrocytes; ElgM) and the alternative pathway (agarose beads) with serumfree mononuclear phagocyte cultures. Then the major complement components of the classical- (C3b, iC3b, C4b,) or alternative (C3b, iC3b,) pathways would bind to the respective activators, as well as prospective binding of the terminal pathway or terminal complement complex (TCC) (C5b-9) on both activators. We first tested binding of a panel of iodine labelled monoclonal antibodies to C3 and to C9 neoantigen present in the TCC and polyclonal C4-C9 antibodies to serum preincubated activators. There was a strong binding of the relevant antibodies, showing strong deposition of C4b on the ElgM and C3-derivates and the TCC on both activators. A key point in the experiments with the serum free mononuclear phagocyte cultures, was to demonstrate biosyn-

TABLE II

Recent findings on complement synthesis by mononuclear phagocytes

Component	Source	Year (reference)	Comment
C3	Peritoneum	—88 (8)	First found functionally active
	Lung	—86 (9)	First found functionally active
C4	Blood	—87 (11)	First found functionally active
	Lung	—87 (11)	First detected and functionally active
C5	Blood	—86 (10)	First found functionally active
	Lung/perit.	—86 (9)/—88 (8)	First detected and functionally active
C6, C7, C8, C9	Blood	—86 (10)	First detected and functionally active
	Lung	—86 (9), —87 (16)	First detected and functionally active
	Peritoneum	—88 (8)	First detected and functionally active

Abbreviation: Peritoneum (perit.)

thesis of protein with affinity for the complement activators. The complement activators were incubated with the phagocytes in the presence of tritiated leucine, and the harvested activators increasingly bound labelled protein from 24 – 72 hours of incubation. Some of the labelled protein was removed from the agarose beads after SDS washing, showing that the protein was bound covalent (C3-derivates?) or noncovalent (C5b-9?) to the beads. There was an increasing binding of anti-C3 and anti-C5-C9 antibodies to both activators upon prolonged coculture as well as binding of anti-C4 to the EIgM (data not shown). The results (Table II) showed that the functional classical-, alternative- and terminal pathway was generated by both monocytes and macrophages from lung and peritoneum. The monocytes seemed to produce more complement than the macrophages as evaluated from greater binding of antibodies and labelled protein to the activators. No detectable binding of anti-S-protein antibodies to the activators was found, showing particle bound TCC devoid of S-protein (C5b-9).

CONCLUDING REMARK

Our (Table II) and previous results (Table I) show that mononuclear phagocytes (monocytes/macrophages) in vitro constitute an independent source of the functional complement system. The in vivo implication probably is local phagocyte secretion of complement components with generation of the activities stemming from complement activation in the tissues including the inflammatory response.

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