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Complement-dependent inhibition of degradation of soluble immune complexes and immunoglobulin aggregates by thioglycollate-stimulated peritoneal macrophages

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Summary. Previous studies have shown that the degradation of soluble immune complexes or aggregates (AIgG) by normal peritoneal macrophages can be enhanced by complement. The enhancement of degradation was shown to be at least in part dependent on the number of C3b molecules bound per complex. The present investigations indicate that the enhanced degradation is not found with thioglycollate-stimulated macrophages, and that at high concentrations of complement, inhibition may even occur. The Fc receptor-mediated degradation of soluble immune complexes and AIgG by stimulated macrophages was at least twice as high as that by normal macrophages. This increase was compatible with the increased number of Fc receptors on the stimulated macrophages. The inhibitory effect of high concentrations of serum, as a complement source, on the degradation of AIgG was dependent on the number of C3b molecules bound per AIgG. Although there was also a two-fold increase in the number of C3b receptor sites on the stimulated macrophages, more than 11 C3b molecules per AIgG40 caused significant inhibition of degradation. This phenomenon may be dependent on shielding of Fc–Fc receptor interaction by varying numbers of C3b molecules per complex.

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INTRODUCTION

Macrophages play an important role in the clearance of circulating immune complexes and thus may prevent immune complex deposition in vessel walls. The mechanism by which macrophages eliminate immune complexes has been studied mainly *in vitro*. These studies have shown that macrophages can bind, internalize and degrade immune complexes and immunoglobulin aggregates via their Fc receptors alone (Leslie & Alexander, 1979; Knutson, Kijlstra & van Es, 1979; Daha & van Es, 1981). Recent investigations have shown that complement can markedly enhance the endocytosis and subsequent degradation of immune complexes (van Snick & Masson, 1978; Kijlstra, van Es & Daha, 1981) and immunoglobulin aggregates by macrophages, an effect which was shown to be dependent upon the ability of the complexes or aggregates to bind and activate the complement system (Kijlstra, van Es & Daha, 1979a, b; Daha & van Es, 1981). Subsequent studies (Daha & van Es, 1982) demonstrated that the number of C3b molecules per aggregate determines the degree of enhancement of degradation by normal peritoneal macrophages; aggregate-bound C3b on the other hand could diminish the Fc-mediated degradation of the aggregates when cellular C3b receptors were functionally inactivated by trypsin. Since the previous studies were

performed with unstimulated macrophages, we have now investigated whether complement-dependent enhancement of endocytosis and degradation of immune complexes also occurs with thioglycollate-stimulated macrophages.

MATERIALS AND METHODS

Immunoglobulin aggregates and immune complexes

Stable ^{125}I-labelled soluble aggregates of heat-treated guinea-pig IgG2 were prepared as described earlier (Kijlstra *et al.*, 1979a). The aggregates used in this study contained approximately 40 IgG2 molecules per aggregate (AIgG40). Immune complexes were formed using bovine thyroglobulin (BTg) as the antigen and immunospecific IgG2 anti-BTg as antibody. Antibodies against BTg were raised in guinea-pigs by intramuscular immunization with BTg in Freund's complete adjuvant. Immunospecific IgG2 anti-BTg was isolated as described previously (Kijlstra *et al.*, 1981). BTg was isolated as reported earlier (Kijlstra *et al.*, 1977) and iodinated with ^{125}I to a specific activity of approximately 0·5 mCi/mg protein. Soluble immune complexes were prepared by incubating a constant amount of 0·5 mg [^{125}I]-BTg/ml with varying amounts of IgG2-anti-BTg (0–6 mg/ml) for 30 min at 37° followed by a 2-hr incubation at 0°. Insoluble complexes were removed from the preparation by centrifugation for 10 min at 5000 ***g*** and the supernatants used to study the effect of Ab:Ag combining ratio on the ability of the macrophages to degrade these complexes.

Degradation studies

Non-stimulated macrophages from normal Hartley strain guinea-pigs were obtained and allowed to adhere to lightly siliconized glass tubes (Kijlstra *et al.*, 1979a). Stimulated macrophages were obtained 5 days after intraperitoneal injection of guinea-pigs with 5 ml of a thioglycollate solution (DIFCO) as described previously (Stewart, Alder & Hibbs, 1978). The cells were washed in Tris-buffered Hanks's medium containing 0·5% BSA (TBH-BSA) and pipetted into lightly siliconized glass tubes to allow the macrophages to adhere to the tubes (10^6 cells/tube; Knutson, Kijlstra & van Es, 1977; Kijlstra *et al.*, 1979a). The percentage of adherent cells was generally 60–70% and was calculated by counting the number of non-adherent cells. All results are expressed as the percentage of complexes degraded by 10^6 adherent cells and each point represents the mean ± SD of six observations. The uptake and subsequent degradation of [^{125}I]-AIgG40, [^{125}I]-AIgG40-C3b, and of ^{125}I-labelled immune complexes by adherent peritoneal macrophages were measured as described previously (Daha & van Es, 1982). Fresh frozen guinea-pig serum (NGPS) or C4-deficient serum was used as a source of complement. [^{125}I]-AIgG40 bearing a varying number of C3b molecules per aggregate were prepared as described (Daha & van Es, 1982). The relative numbers of binding sites for AIgG40 and for tetrameric C3b (AC3b) were determined by incubation of 2×10^6 adherent macrophages with varying amounts of [^{125}I]-AIgG40 (2–200 fmol) or [^{125}I]-AC3b (2–200 fmol) in 0·2 ml TBH-BSA for 24 hr at 4° (Daha & van Es, 1982). The number of binding sites was calculated by Scatchard analysis.

RESULTS

To determine the effects of complement on the degradation of soluble [^{125}I]-AIgG40 by normal and thioglycollate-stimulated cells, 25 fmol quantities of [^{125}I]-AIgG40 were incubated with adherent macrophages in the presence and absence of varying concentrations of NGPS for 1 hr at 37°. In agreement with previous results, NGPS enhanced the degradation of [^{125}I]-AIgG40 by normal peritoneal macrophages (Fig. 1). In the absence of NGPS, thioglycollate-stimulated macrophages degraded a higher percentage of [^{125}I]-AIgG compared to unstimulated macrophages (65%). In contrast to normal macrophages, serum did not stimulate, rather it inhibited the degradation of AIgG by stimulated macrophages. This phenomenon was dependent on an intact complement system because C4-deficient guinea-pig serum did not inhibit the degradation of [^{125}I]-AIgG40 and the inhibitory effect could however be restored by the addition of purified C4 to the C4-deficient serum (Table 1).

To explore further the difference in complement-dependent degradation of [^{125}I]-AIgG40 by stimulated and unstimulated peritoneal macrophages, a kinetic experiment was performed. Macrophages were incubated with [^{125}I]-AIgG40 in the presence or absence of 20% NGPS and the percent degradation determined at timed intervals. There was a time-dependent increase in degradation of [^{125}I]-AIgG40 in medium alone by both normal and stimulated macrophages (Fig. 2); the latter were about twice as active as normal macrophages at all the points. The presence of 20% NGPS

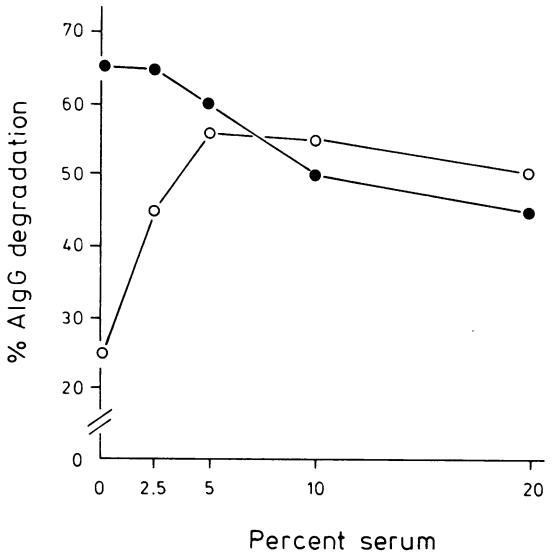

Figure 1. Degradation during 1 hr at 37° of soluble IgG2 aggregates ([^{125}I]-AIgG40) by non-stimulated (○) and thioglycollate (●) stimulated macrophages in TBH-BSA alone or in TBH-BSA containing various concentrations of normal guinea-pig serum.

during the incubation period resulted in enhanced degradation of [^{125}I]-AIgG40 by normal macrophages but caused a significant degree of inhibition by the stimulated macrophages, which however was still higher than the degradation of AIgG40 by normal peritoneal macrophages in the absence of complement.

In order to define further the role of complement in the degradation of soluble immune complexes by stimulated macrophages, the capacity of normal and stimulated macrophages to degrade [^{125}I]-BTg complexes prepared at varying antibody to antigen ratios was determined in the presence and absence of 20%

Table 1. Effect of complement on the degradation of 25 fmol [^{125}I]-AIgG40 by thioglycollate-stimulated macrophages after 1 hr at 37°

	% Degradation*
AIgG+TBH-BSA	56·2±4·1
AIgG+20% NGPS	39·1±3·9
AIgG+C4-def. GPS	55·1±4·9
AIgG+C4-def. GPS+C4	41·3±4·3
AIgG+TBH-BSA+C4	57·3±5·1

* Expressed as mean±1 SD.

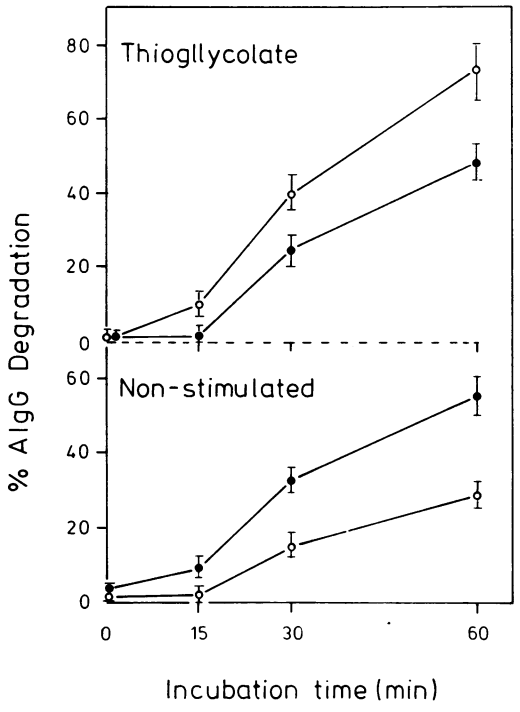

Figure 2. Time-dependent degradations of soluble [^{125}I]-AIgG40 by non-stimulated (*lower panel*) and thioglycollate stimulated (*upper panel*) macrophages in the absence (○) and presence (●) of 20% normal guinea-pig serum.

NGPS (Figs 3, 4). The presence of 20% NGPS enhanced the capacity of normal macrophages to degrade immune complexes, prepared at Ab:Ag ratios of 3·5 and 7·0, but caused a slight inhibition of degradation of these complexes by the stimulated macrophages ($P=0·05$).

Because it has been shown previously (Daha & van Es, 1981) that the complement-dependent degradation of soluble complexes is mediated for the major part by the interaction of complex-bound C3b and cellular C3b receptors, AIgG40 was prepared bearing 0, 5, 11, 15 and 20 molecules of C3b per aggregate and incubated with normal and stimulated macrophages in TBH-BSA for 1 hr at 37°. As noted before (Daha & van Es, 1982) (Fig. 4), an enhancement of degradation by normal macrophages was seen dependent on the number of C3b molecules per AIgG40. On the other hand no enhancement of degradation was seen in the case of stimulated macrophages, and 15 or 20 molecules of C3b/AIgG40 caused inhibition of degradation.

To determine the number of Fc- and C3b-binding

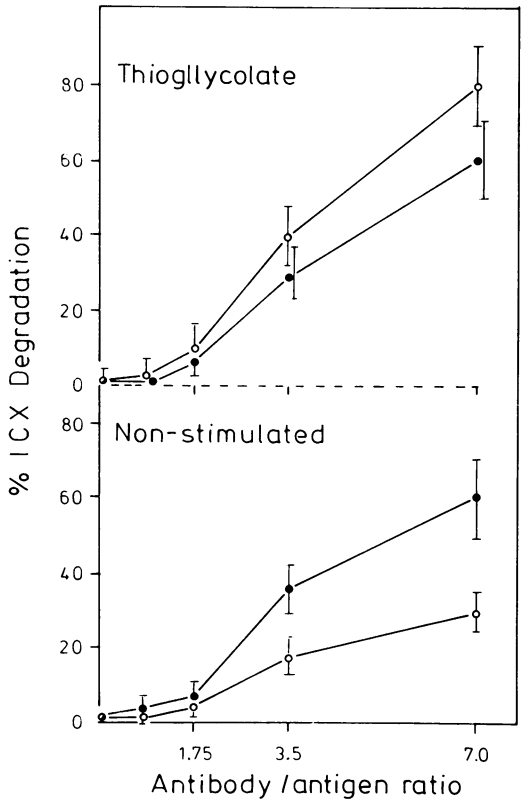

Figure 3. Degradation of soluble immune complexes prepared at various Ab: Ag ratio by non-stimulated (*lower panel*) and thioglycollate-stimulated (*upper panel*) macrophages in the absence (○) and presence (●) of 20% normal guinea-pig serum.

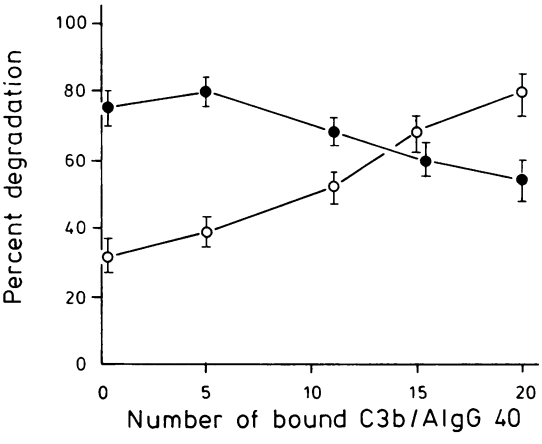

Figure 4. Degradation of AIgG40 bearing 0, 5, 11, 15 and 20 molecules of C3b by normal (○) and thioglycollate-stimulated macrophages (●).

Table 2. Number of binding sites per macrophage for AIgG40 and tetrametic C3b (AC3b) as calculated by Scatchard analysis

Macrophages	Number of AIgG40 binding sites	Number of AC3b binding sites
Non-stimulated	150,300 ± 6080	61,200 ± 4117
Thioglycollate	378,213 ± 8091	133,616 ± 5213

sites per cell, stimulated and normal macrophages were incubated with varying concentrations of [^{125}I]-AIgG40 or [^{125}I]-AC3b for 20 hr at 4° and the number of binding sites determined. The results (Table 2) indicate that thioglycollate-stimulated macrophages have 2·5 and 2·0 times more receptors for AIgG40 and AC3b, respectively, than do normal macrophages.

DISCUSSION

Previous investigations have shown that the enhancing effect of complement on the degradation of soluble immune complexes (Kijlstra *et al.*, 1981) and soluble immunoglobulin aggregates (Kijlstra *et al.*, 1979a, b; Daha & van Es, 1981) is determined at least in part by the binding of C3b in the aggregates or immune complexes to functional C3b receptor sites on phagocytes. Subsequent studies (Daha & van Es, 1982) have indicated that the enhancement of degradation of soluble AIgG is dependent on the number of C3b molecules per aggregate. The present studies were initially performed to determine the effect of different numbers of Fc receptors per cell on the degradation of soluble immune complexes and immunoglobulin aggregates. When thioglycollate-stimulated macrophages were used, it was observed that the Fc receptor-mediated degradation in medium alone was indeed clearly increased when the number of Fc receptors on the phagocytes had been increased by thioglycollate stimulation (Fig. 1, Table 2).

In contrast to the findings with normal peritoneal macrophages however, the enhancing effect of complement on the degradation of immune complexes and AIgG was not found when thioglycollate-stimulated macrophages were used. Even inhibition was observed at high concentrations of serum (Fig. 1). That this effect was complement-dependent was shown by experiments in C4-deficient serum in which the classical pathway is impaired (Table 1). The inhibitory effect of complement on the degradation of AIgG by

stimulated macrophages was not related to an absence of C3b-receptors on these cells, since these macrophages possessed increased numbers of C3b receptors as determined by binding of [^{125}I]-AC3b (Table 2). Experiments with AIgG40 that bore varying numbers of C3b molecules demonstrated that more than 11 C3b molecules per AIgG40 inhibited the degradation of these aggregates by stimulated macrophages. Since it has been shown previously that the binding step is the rate-limiting step in the processing of soluble AIgG and immune complexes (Kijlstra *et al.*, 1979b) it is likely that C3b influences this step. Possibly, when macrophages have a high Fc-receptor density, excess C3b molecules on one aggregate sterically hinder adjacent Fc receptors from binding additional aggregates, causing inhibition of degradation. When immune complexes were used instead of AIgG, the inhibitory effect of complement on the degradation of these complexes by stimulated macrophages was less pronounced. An explanation for this phenomenon may be that the soluble immune complexes used were more heterogeneous in size and composition (Kijlstra *et al.*, 1981) than AIgG aggregates (Knutson *et al.*, 1979; Kauffmann, van Es & Daha, 1979) and that complexes are formed with a varying number of C3b molecules. The different numbers of C3b molecules per complex may induce variable degrees of inhibition of degradation. Taken together, the results from this study imply that, depending on the capacity of complexes to bind and activate complement, populations of complexes are formed which interact with phagocytes with different avidity. In addition, certain populations of phagocytes may be more efficient in the clearance of certain populations of complexes. It is also possible that complement may, by this mechanism, deviate complexes to specialized organs for the clearance of complexes of specific types (Atkinson & Frank, 1974). These hypotheses require further investigation but can be approached with stable complexes bearing different numbers of C3b molecules per complex.

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