Effects of Polymeric C3, C3b and iC3b on Neutrophil Expression of CD11b and CD18

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Key Words

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Introduction

Complement activation in vivo in rats is known to induce neutrophil (PMN) activation and endothelial damage in the lung and other organs [1]. The mechanisms for this injury are thought to involve C3, PMN-CD11b/CD18 selectin and endothelial adhesion molecules 1-CAM 1 and P-selectin [2, 3]. To clarify these events in vitro, we have purified C3, C3b and iC3b. The effect of C3 and its products were tested in human PMN expression of the B2-selectin, CD11b/CD18.

Results

Since the CD 11b/CD 18 complex is known to possess binding sites for iC3b, we explored the role of C3, C3b and iC3b on CD11b/CD18 expression with human PMNs. C3, C3b and iC3b were purified from human plasma by precipitation with polyethylene glycol, anion exchange chromatography and gel filtration [4]. C3 was further purified to remove traces of C5, IgE and factor 11. These procedures yielded C3 which was more than 98% pure. C3b was prepared from C3 by treatment with 1 µg trypsin/mg C3. The reaction was stopped with soybean trypsin inhibitor. iC3b was prepared from C3b by treatment with 9 µg factor H and 5 µg factor I/mg C3b. Both C3b and iC3b were further purified by gel filtration in Biogel A 0.5 m (BioRad, Richmond, Calif, USA). Purified C3, C3b and iC3b were cross-linked with dimethyl superimide [5]. The effects of native (non-cross-linked) and cross-linked C3, C3b and iC3b were studied by cytofluorimetry on uptake of C3b as well as expression of CD11b/CD18. Cross-linked C3b bound to PMNs with five times greater fluorescent intensity than native C3b.

To study CD11b/CD18 upregulation, PMNs were stimulated with FMLP (10⁻⁵ M) and showed a 111% increase of both CD 11b and CD18. Nonstimulated PMNs were incubated with native and cross-linked (1.25, 2.5 and 5.0 µg) C3, C3b and iC3b. Dose-response increases of CD18 > CD11b were found with cross-linked C3b and iC3b. Cross-linked iC3b increased CD18 expression by 8,

56 and 126%. Cross-linked C3 demonstrated a dose response of CD 11b, but not CD18. Cross-linked C3 increased expression of CD11b by 2, 43 and 132% above baseline. Native C3, C3b and iC3b at the highest dose (5 µg) showed little or no increase in CD11b/CD18. We have shown that cross-linked iC3b and C3b are both bound to PMNs and increase CD11b/CD18 expression. Non-cross-linked C3, C3b and iC3b have little or no effect on PMN uptake or upregulation of CD11b/CD18. Further studies on the effect of C3, C3b and iC3b on PMN-endothelial adherence are in progress.

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References

Mulligan MS, Smith CW, Anderson DC, Todd RF, Miyasaka M, Tamatani T, Issekutz TB, Ward PA: Role of leukocyte adhesion molecules in complement-induced lung injury. *J Immunol* 1993;150:2401-2406.

Mulligan MS, Polley MJ, Bayer RJ, Nunn MF, Paulson JC, Ward PA: Neutrophil-dependent acute lung injury. Requirement for P-selectin (GMP-140). *J Clin Invest* 1992;90:160-167.

Foreman KE, Vaporciyan AA, Bonish BK, Jones ML, Johnson KJ, Glovsky MM, Eddy SM, Ward PA: C5a induced expression of P-selectin in endothelial cells. *J Clin Invest* 1994; 94:1147-1155.

Pangburn MK: A fluorimetric assay for native C3. *J Immunol Methods* 1987;102:7-14.

Fearon DT, Collins LA: Increased expression of C3b receptors in polymorphonuclear leucocytes induced by chemotactic factors and purification procedures. *J Immunol* 1983; 130:1.

Foreman KE, Glovsky MM, Warner RL, Horvath SJ, Ward PA: Comparative effect of C3a and C5a on adhesion molecule expression on neutrophils and endothelial cells. *Inflammation* 1996;20:1-9.

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