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

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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 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 CD11b/CD18 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 superimidate [5]. The effects of native (non-cross-linked) and cross-linked C3, C3b and iC3b were studied by cytfluorimetry 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 III% increase of both CD11b 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 CD11b > 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 CD1lb, but not CD18. Cross-linked C3 increased expression of CD1lb by 2, 43 and 132% above baseline. Native C3, C3b and iC3b at the highest dose (5 µg) showed little or no increase in CD1lb/CD18. We have shown that cross-linked iC3b and C3b are both bound to PMNs and increase CD1lb/CD18 expression. Non-cross-linked C3, C3b and iC3b have little or no effect on PMN uptake or upregulation of CD1lb/CD18. Further studies on the effect of C3, C3b and iC3b on PMN-endothelial adherence are in progress.

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