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

R.L. Warner a
M.M. Glovsky b
M.K. Pangburn c
P.A. Ward a

aDepartment of Pathology, University of Michigan, Ann Arbor, Mich., bAsthma and Allergy Center, Huntington Memorial Hospital, Pasadena, Calif., and cHealth Science Center, Department of Biochemistry, University of Texas, Tyler, Tex., USA

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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 I. 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 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 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 > CD18 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 CD1b, but not CD18. Cross-linked C3 increased expression of CD1b by 2, 43 and 132% above baseline. Native C3, C3b and iC3b at the highest dose (5 µg) showed little or no increase in CD1b/CD18. We have shown that cross-linked iC3b and C3b are both bound to PMNs and increase CD1b/CD18 expression. Non-cross-linked C3, C3b and iC3b have little or no effect on PMN uptake or upregulation of CD1b/CD18. Further studies on the effect of C3, C3b and iC3b on PMN-endothelial adherence are in progress.

References


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