Peptidoglycan Induces Mobilization of the Surface Marker for Activation Marker CD66b in Human Neutrophils but Not in Eosinophils.

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Peptidoglycan Induces Mobilization of the Surface Marker for Activation Marker CD66b in Human Neutrophils but Not in Eosinophils

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Peptidoglycan from Staphylococcus aureus mobilized CD66b in human neutrophils but did not upregulate surface activation markers in eosinophils. In addition, Toll-like receptor 2, implicated in the recognition of peptidoglycan, was detected on the surface of resting neutrophils but not on eosinophils. These findings suggest roles for neutrophils but not eosinophils in innate recognition of peptidoglycan.

Staphylococcus aureus is a major pathogen in endocarditis, septicemia, infectious arthritis, and nosocomial infections (9). On the other hand, S. aureus often colonizes skin and mucosal surfaces without causing disease in asymptomatic carriers (9). Therefore, it is important for the host defense to have the ability to adapt its immune response, with an increased immunosurveillance and propensity to react during states of colonization and a rapid recognition and destruction of the bacteria during invasion of tissues. The cell wall of S. aureus is composed mainly of peptidoglycan (PG) (50 to 60% by weight) (8). PG has several biological effects, such as induction of cytokines and tissue factor expression in human monocytes (12, 13). Other products derived from S. aureus, some of them acting as superantigens, have been suggested to play pathophysiologic roles in allergic inflammation (1).

Recent years of research have revealed several receptors for the innate recognition of bacterial cell wall components by the immune system. Toll-like receptor 2 (TLR-2) can recognize PG, while TLR-4 together with a combination of other molecules, among them CD14, are held responsible for the recognition of lipopolysaccharides of gram-negative bacteria (3, 16).

Neutrophils are known to play important roles in the host defense against bacterial invasion. Eosinophils, on the other hand, may be important in the defense against parasitic infestation, but this issue is yet to be settled (2, 4, 19). In addition, eosinophils are involved in diseases with allergic inflammation such as asthma (19). At present, there are conflicting data concerning the presence of TLR-2 and -4 on both neutrophils and eosinophils (14, 15).

The aim of this study was to investigate whether PG can activate human neutrophils and eosinophils. The method used has been described previously and reflects different grades of cellular activation in neutrophils and eosinophils by the detection of increased expression of surface antigens (7, 11). In addition, the surface expression of TLR-2 and -4 on neutrophils and eosinophils was investigated.
In neutrophils, PG caused a dose- and time-dependent increase in the mobilization of CD66b to the cell surface compared with controls (Fig. 1). The mobilization was enhanced in the presence of cytochalasin B compared with the response in the absence of this compound (data not shown). A spontaneous increase in CD66b, although weaker, was seen in the absence of PG. As a consequence, at each time point the mean fluorescence intensity of stimulated cells was related to that of resting cells. To compare the activating potency of PG, neutrophils were incubated with the well-characterized inducer of CD66b mobilization, fMLP, in parallel (7). PG induced mobilization of CD66b on the order of magnitude of that seen in the presence of fMLP (Fig. 1C).

CD66b is a glycosyl phosphatidylinositol-anchored membrane protein belonging to the immunoglobulin-like subfamily of carcinoembryonal antigens (17). It is present in the membrane of specific and gelatinase-containing granules of neutrophils, and stimuli such as fMLP induce its mobilization to the cell surface (4, 5, 7). Cross-linking of CD66b results in cellular responses, for example, respiratory burst and increased adhesion (10). In addition, CD66b has been suggested to serve as a receptor for galectin 3 (5).
PG (100 μg/ml) did not induce significant mobilization of selected surface markers in human eosinophils—i.e., CD11b, CD63, and CD66b during 40 min of incubation or CD44 and CD69 after 4 and 18 h of incubation, respectively (Fig. 2). In addition, eosinophils were coincubated with the eosinophil-activating cytokine interleukin 5 (IL-5) (1 nM) and PG (100 μg/ml), but no increased mobilization of surface activation markers was observed. To exclude paradoxical effects from lower concentrations of PG, cells were incubated in the presence of PG at 1 and 10 μg/ml (in the absence or presence of IL-5). However, nor did these lower concentrations of PG cause mobilization of CD44 or CD69 (data not shown). As a control, some cells were stimulated with the calcium ionophore A23187 (1 μM) for 20 min, to assure that the cells were responsive and that the surface markers could indeed be mobilized.

TLR-2 and, to a lesser extent, TLR-4 were detected on the surface of neutrophils by flow cytometry in the present study (Fig. 3a). The higher expression of TLR-2 and the lower expression of TLR-4 are in agreement with that reported in a recent study (13). Incubation of neutrophils with PG (100 μg/ml) for 30 min did not change the amount of TLR-2 or TLR-4 on the cell surface (data not shown). Neither TLR-2 nor TLR-4 could be detected on the surface of eosinophils (Fig. 3b), and exposure to PG (100 μg/ml) for 30 min did not result in the appearance of TLR-2 or TLR-4 on the surface of eosinophils. In a recent study, gene expression of both TLR-2 and TLR-4 was detected in eosinophils (14). However, the
presence of the receptors on a protein level was not investigated. In another investigation, none of these receptors could be detected on the surface of eosinophils, nor did they respond to lipopolysaccharides while PG was not investigated (15).

Other cell wall components may be recognized by eosinophils. Recently, these cells were shown to respond to several exotoxins (SEA, SEB, SEC, and toxic shock syndrome toxin 1) derived from S. aureus that can serve as superantigens (18). Therefore, eosinophils may possess the ability to interact with S. aureus through innate recognition in vivo. This may be of importance during allergic inflammation (1).

In conclusion, the present study suggests roles for neutrophils but not eosinophils in innate recognition of bacterial PG.

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