Lipopolysaccharide needs soluble CD14 to interact with TLR4 in human monocytes depleted of membrane CD14

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Toll-like receptors recognize specific patterns of microbial components and regulate the activation of both innate and adaptive immunity. TLR4 recognizes lipopolysaccharide (LPS) in monocytes/macrophages with the help of other molecules like CD14 and MD-2, which indicates that the functional LPS receptor forms a large complex. The functional relationship between the components has been the subject of debate, as have the modifications induced by the ligand in the expression of some of these components. Moreover, as for other members of this family of receptors, the possible direct interaction of receptors and their ligands is a matter of discussion. In this paper we address the question of whether the expression of some of the components influences the expression of the rest. Human monocytes in which CD14 has been downregulated through interference in the turnover of the molecule at the Golgi level, show normal membrane TLR4 expression, when compared with control cells. On the other hand, LPS alters membrane TLR4 expression by monocytes devoid of membrane CD14 only in the presence of human serum. The effect of serum is blocked by anti-CD14 monoclonal antibodies, which strongly suggests a functional role for soluble CD14/LPS complexes in the interaction with TLR4. Our data add information on the relationship between the components of the LPS receptor and the characteristics of the interaction of LPS and TLR4 in cells devoid of membrane CD14.

Anti-inflammatory cytokines induce lipopolysaccharide tolerance in human monocytes without modifying toll-like receptor 4 membrane expression

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Toll-like receptor 4 (TLR4) participates in innate immunity by detecting lipopolysaccharides (LPS) of Gram-negative bacterial cell walls. TLR4 macrophage expression in mice is modulated by LPS. This fact constitutes, at least partially, the molecular basis for LPS tolerance. Very recently, the effect of interferon-gamma (IFN-gamma), a pro-inflammatory cytokine, has been described on TLR4 membrane expression of human monocytes. IFN-gamma up-regulates TLR4 expression and antagonizes the LPS-induced TLR4 down-regulation. These data prompted us to study the expression of membrane TLR4 in human mono- cytes in which LPS tolerance was induced by LPS and by anti-inflammatory cytokines [interleukin-10 (IL-10) and transforming growth factor beta1 (TGFbeta1)]. Data concerning this latter model, and more specifically, the effect of anti-inflammatory cytokines over TLR4 expression, are not available at present. We show here that membrane TLR4 expression in human monocytes falls after LPS exposure. The effect was prolonged for 12 h, but then expression returned to normal levels. The incubation of human monocytes with IL-10, TGFbeta1 or a mixture of both induces no alterations in membrane TLR4 expression. However, these cytokines are able to substitute the tolerizing LPS exposure in order to induce LPS tolerance. Our data help to achieve a better understanding of the way cytokines control the cellular expression of TLR.

A New Combined Test with Flowcytometric Basophil Activation and Determination of Sulfidoleukotrienes Is Useful for in vitro Diagnosis of Hypersensitivity to Aspirin and other Nonsteroidal Anti-Inflammatory Drugs

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Background: We assessed whether nonsteroidal anti-inflammatory drugs (NSAIDs) may provoke blood basophil activation in vitro in aspirin- and NSAID-hypersensitive patients, as detected by a flowcytometric technique using the CD63 marker - flowcytometric basophil activation test (FAST) assay - in addition to the sulfidoleukotriene (sLT) release - the cellular allergen stimulation test (CAST). Methods: Sixty aspirin- and/or NSAID-hypersensitive patients were studied. Thirty control patients without history and negative provocation challenge were also included. The percentage of activated basophils after in vitro stimulation with NSAIDs at 3 different concentrations was evaluated by an anti-CD63 phycoerythrin conjugate (FAST assay) and the amount of sLTs released in the cell supernatant by ELISA (CAST assay). Results: For aspirin, the FAST indicated a sensitivity of 41.7%, a specificity of 100%, a positive predictive value of 100% and a negative predictive value of 99.4%; for paracetamol 11.7 and 100%, for metamizol 15 and 100%, for diclofenac 43.3 and 93.3%, and for naproxen 54.8 and 74.1%. Many patients showed positive tests to more than 1 NSAID. When considering the first 4 NSAIDs, the global sensitivity increased to 66.7%, while the specificity remained at 93.3%. The addition of the CAST results still increased the sensitivity up to 73.3%, but with a decrease of the specificity to 71.4%. Conclusions: The FAST shows a high percentage of positive reactions, which may reach 60-70% when 4 NSAIDs are tested and even 88% when the test is performed within 1 month of the last clinical drug exposure and reaction. The test has a high specificity above 90%. The addition of sLT determinations yields additional information in a few isolated cases. It is suggested that this test, when properly used, may help avoid some cumbersome and dangerous provocation challenges.