

Immunostimulatory properties of dendrimers multivalently presenting muramyl dipeptide

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Objectives: Many pathogens will only be efficiently neutralized by the induction of cell-mediated immunity, and with the enhanced use of subunit-vaccine approaches there is a strong need for the development of efficient and safe Th1-biasing adjuvants. Pathogen-associated molecular patterns (PAMPs) are evolutionarily conserved microbial structures, generally composed of repeated molecular units of small size, and recognized by pattern-recognition receptors (PRRs). Binding of PAMPs to certain PRRs induces dendritic cells to express costimulatory molecules and inflammatory cytokines, enabling an inductive antigen-presentation. This project aims at using small PAMP-units to prepare molecularly defined adjuvants for the targeted delivery of antigens in an optimally immunostimulatory manner.

Methods: We investigated whether the repetitive structure of PAMPs may be mimicked by multivalent presentation of PAMP minimal motifs on the surface of monodisperse hyperbranched globular synthetic polymers, dendrimers. Two sizes of polypropylene-imine (PPI) and polyamido-amine (PAMAM) dendrimers were conjugated with muramyl dipeptide (MDP), the minimal essential motif of peptidoglycan (PGN), to yield constructs presenting a theoretical number of 16 and 32 MDP molecules, respectively. These conjugates were tested for cytotoxicity, and for their ability to induce cytokines and upregulate MHC and costimulatory molecules in porcine peripheral blood mononuclear cells.

Results: Both MDP-PPI and MDP-PAMAM conjugates induced a considerable, dose-dependent production of IL-12 p40, IL-1 β and IL-6, in contrast to unmodified dendrimers and free MDP at equivalent concentrations. The MDP-PAMAM conjugates were particularly efficient inducers of all three cytokines, inducing IL-12 p40, IL-1 β and IL-6 to the same level as PGN. Interestingly, whereas PGN induced a considerable IL-10-production, no IL-10 was induced by the MDP-conjugated dendrimers. By flow cytometry, enhanced cell death was detected in response to unmodified PPI-dendrimers, whereas none of the MDP-conjugated dendrimers showed any detectable cytotoxicity. The monocytes showed an upregulation of B7 and MHC class II after 24 hours of culture in medium. However, after culture with PGN or MDP-conjugated dendrimers, the monocytes had down-regulated both markers to a degree that corresponded with the magnitude of cytokine production.

Conclusion: These results indicate that it is possible to employ dendrimers in the synthesis of molecularly defined globular PAMP-mimics or "artificial microbes" with controlled immunostimulatory properties.