

protection with the present invention would not have been expected by the art prior to the cited disclosures.

[0025] Furthermore, U.S. application Ser. Nos. 09/257,188 and “number not yet designated” (docket PMS254806) disclose penetration enhancers (e.g., removal of superficial layers above the dermis, micropenetration to above the dermis) and targeting of complexed antigen and/or adjuvant in the context of transcutaneous immunization.

SUMMARY OF THE INVENTION

[0026] An object of the invention is to provide an improved system for immunization or vaccination in an organism in need of such treatment. Transcutaneous immunization can involve simple application of a formulation comprised of at least one antigen, adjuvant, polynucleotide, and combinations thereof to induce an immune response. This immune response can be enhanced by hydration of the site where the formulation is applied, superficial penetration or micropenetration at that site, formation of complexes between or among components of the formulation, or the addition of other physical manipulations during immunization or chemical additives to the formulation. But such enhancement is not required to evoke a useful antigen-specific immune response. This delivery system provides simple application of a formulation comprised of at least antigen or adjuvant, or of polynucleotide encoding antigen or adjuvant, to intact skin of an organism which induces at least a specific response against the antigen by the organism's immune system.

[0027] It is a particular object of the invention for transcutaneous immunization to provide a protective immune response for prophylactic or therapeutic treatment.

[0028] Examples of such responses include vaccination that protects against subsequent antigenic challenge or pathogenic infection, or a reduction in the number and/or severity of symptoms that are associated with an or infectious disease.

[0029] In particular, the invention may promote contact between antigen and immune cells. For example, antigen presenting cells (e.g., Langerhans cells in the epidermis, dermal dendritic cells, dendritic cells, follicular dendritic cells, B cells, macrophages) with antigen, adjuvant, polynucleotide, or a combination thereof may enhance activation of the antigen presenting cell and/or presentation of antigen. The antigen presenting cell would then present the antigen to a lymphocyte. In particular, the antigen presenting cell may migrate from the skin to the lymph nodes, and then present antigen to a lymphocyte, thereby inducing an antigen-specific immune response. Moreover, the formulation may directly contact a lymphocyte which recognizes antigen, thereby inducing an antigen-specific immune response.

[0030] In addition to eliciting immune reactions leading to activation and/or expansion of an antigen-specific B and/or T cell population, including a cytotoxic T lymphocyte (CTL), another object of the invention is to positively and/or negatively regulate components of the immune system by using the transcutaneous immunization system to affect antigen-specific helper (Th1 and/or Th2) or delayed-type hypersensitivity (DTH) T-cell subsets through the use of different classes of skin-active adjuvants. The desired immune response is preferably systemic or regional (e.g., mucosal), but it is primarily not an allergic reaction, dermatitis, eczema, psoriasis, or other atopic skin reactions.

[0031] The invention may be practiced without perforation of the intact skin. But the invention may also include applying the formulation to skin with physical energy, electrical

energy, sonic energy, or combinations thereof used to perforate the stratum corneum to reach the outer layer of the epidermis. Optionally, the formulation may include chemical penetration enhancers, viral particles, whole or intact cells, Liposomes, proteosomes, chemical transfectants, materials to promote skin hydration, or combinations thereof. Hydrating the skin at the application site or recruiting antigen presenting cells to the application site may enhance the immune response.

[0032] In contrast to the expectations of the art, our delivery system provided by transcutaneous immunization is capable of achieving efficient delivery of at least antigen and/or adjuvant through the skin to the immune system. This may be accomplished with skin-active adjuvants that induce a systemic and/or regional immune response without the harmful side-effects that were expected for such potent activators of the immune system.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] FIG. 1, panels A-B, shows the CT-specific antibody responses in BALB/c mice immunized transcutaneously with cholera toxin (CT). The ordinate of panel A is exponentially scaled and arrows indicate the 8 and 18 week time points. Panel B displays the antibody titers induced after the 18 week boost on a linear scale. An asterisk (*) denotes a statistically significant increase ($p < 0.05$) in anti-CT antibody titer between 18 and 23 weeks.

[0034] FIG. 2, panels A-B, shows mortality in a population of C57BL/6 mice that have been immunized with CT by the transcutaneous route and then intranasally challenged with native toxin three weeks after (A) one or (B) two rounds of immunization. In both trials, survival was significant at the $p < 0.05$ level (Fisher Exact). The number of mice per group is indicated in parentheses (total survivors/number of mice in study).

[0035] FIG. 3, panels A-F, shows serum (A and D) and mucosal (lung in B and E; stool in C and F) antibody responses to CT after transcutaneous immunization.

[0036] FIG. 4, panels A-D, shows serum antibody responses induced by oral (panels A and B) or transcutaneous (panels C and D) exposure to CT. Results shown are measurements from the five individual animals (hollow squares for panels A and C; hollow circles for panels B and D). Solid symbols indicate the geometric mean value for each cohort of animals. An asterisk (*) denotes the mean value detected in prebled serum of the mice.

DESCRIPTION OF PREFERRED EMBODIMENTS

[0037] The transcutaneous immunization system of the present invention can deliver antigen to the immune system through the stratum corneum without physical or chemical penetration to the dermis layer of the skin. This delivery system induces an antigen-specific immune response. Use of skin-active adjuvants is preferred. Although perforation of intact skin is not required, superficial penetration or micropenetration of the skin can act as an enhancer; similarly, hydration may enhance the immune response. This system can induce antigen-specific immune effectors after epicutaneous application of a formulation containing one or more antigen and adjuvant. The formulation may initiate processes such as antigen uptake, processing, and presentation; Langerhans cell activation, migration from the skin to other immune