Mucosal immunoadjuvant activity of liposomes

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SUMMARIZING DISCUSSION
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Mucosal surfaces are sites of entry for many pathogens, such as airborne viruses. These surfaces are protected against infections by the mucosal immune system, involving s-IgA as a major humoral defense factor. Conventional vaccination strategies, generally involving parenteral administration of antigen, rarely induce mucosal immunity. Therefore, new-generation vaccines should aim at the induction of a wide range of immune responses, including a mucosal s-IgA response. Using influenza and measles virus antigen in a murine system, we set out to pursue this goal by exploring an alternative i.n. route of immunization in conjunction with the use of liposomes as an immunoadjuvant system.

Liposomes in an intranasal influenza subunit vaccine

Different antigens have been tested in one or another liposomal formulation, and in many cases, an enhancement of immunogenicity has been observed (6, 52, 146). Few studies, however, have explored the use of liposomes for potentiation of mucosal immune responses via local administration to the respiratory tract. In Chapter 2, we show that incorporation of influenza subunit antigen in liposomes markedly increases the mucosal immunogenicity of the antigen: liposomes stimulate the serum IgM, and IgG antibody responses and, more importantly, induce a mucosal s-IgA response in the respiratory tract. In addition to s-IgA, high levels of IgG antibodies are found in pulmonary secretions (Chapter 3). The IgG subclass distribution shows a preferential induction of IgG1 in serum and pulmonary secretions, however, IgG2a and IgG2b are also induced to considerable levels. I.n. immunization with the liposomal influenza subunit antigen induces local synthesis of IgA and IgG antibodies, as revealed by the presence of specific antibody-secreting cells in the lungs and lung-associated lymph nodes (Chapter 3). This pool of antibody may contribute to protection, in addition to IgG transudated from the serum.

The systemic and local antibody responses elicited by the i.n. liposomal subunit antigen were compared with responses induced by an infection with influenza virus, which induces optimal antibody responses, and parenteral (s.c.) injection of subunit antigen alone, representing the conventional route of immunization in humans. I.n. immunization with the liposomal subunit antigen and infection, but not s.c. immunization with the subunit antigen alone, induces an s-IgA response in the respiratory tract (Chapter 3). This response is most prominent in the lungs. I.n. immunization with the liposomal subunit antigen is as efficient as infection or s.c. immunization in the induction of serum IgG antibody production (Chapter 3). Only at a low dose of antigen the s.c. immunization protocol appears to be more effective than i.n. immunization in induction of serum IgG. This is likely
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to be the result of an ineffective delivery of antigen to the lower respiratory tract, in the i.n. immunization protocol. Studies by Yetter have shown that, in the i.n. administration protocol as used in this study, only about 20% of the inoculum reaches the lungs. At the same time, the (increased) antibody responses observed after i.n. immunization with the liposomal antigen are most likely initiated in lymphoid tissues associated with the lung (Chapters 2, 5 and 6). Therefore, we conclude that the i.n. immunization protocol is as efficacious as parenteral injection of subunit antigen alone in the induction of systemic antibody responses. However, further optimization of the delivery of the liposomal antigen to the lungs is desirable and may be achieved by inhalation of an aerosolized liposomal formulation (125).

The liposomal subunit formulation described in this study provides a promising alternative to the influenza subunit vaccines currently used in human vaccination. The liposomal vaccine could be administered by inhalation, as discussed above, thus avoiding i.m. injection. It is clear that such an approach would provide a more readily acceptable route of administration for the annual flu vaccination.

Protective immunity conferred by an i.n. liposomal influenza subunit antigen

Studies of Renegar and Small (119, 120) have demonstrated that protection of the upper respiratory tract is mediated by local s-IgA antibody. Accordingly, we observed a positive correlation between the presence of nasal s-IgA antibodies, induced by infection, and protection of the nasal mucosa against infection with influenza (Chapter 4). Although the i.n. immunization protocol elicits significant production of s-IgA antibodies in the nasal mucosa (Chapter 2), in the study presented in Chapter 4 the nasal s-IgA antibody titers just fall short of protective levels. Optimization of the induction of s-IgA antibody synthesis in the nasal mucosa is therefore desirable and may include the use of other mucosal adjuvants, such as cholera toxin B or trace amounts of the holotoxin, incorporated in or combined with the liposomal system (144). Our studies further show that, i.n. immunization with liposome-supplemented influenza virus subunit antigen like conventional immunization with subunit antigen alone, confers complete protection from a lethal challenge with homologous virus (Chapter 2 and 4). Both immunization protocols prevent viral pneumonia by inducing high levels of serum IgG, which presumably protects the lung by transudation from the circulation (88, 117).
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Liposomes in an intranal measles virus vaccine

It has become clear from our studies that the immunoadjuvant activity of liposomes is not restricted to influenza virus subunit antigen. In Chapter 5, it is shown that immunization of the respiratory tract of mice with an inactivated measles virus preparation supplemented with liposomes induces high and persisting levels of antigen-specific serum IgG and local s-IgA antibodies. Although (current) live attenuated measles virus vaccines are highly efficacious, an inactivated vaccine has the clear advantage of being more stable, and further offers the possibility to vaccinate young children in the presence of maternal antibodies (110). In this respect, it is interesting to note that maternal antibodies would not likely interfere with virus replication when the (attenuated) virus vaccine is administered via the mucosa (154). Although inactivated vaccines offer many advantages, there is reason for some concern as well. First, atypical measles virus infection has been observed in vaccinees administered an inactivated measles virus vaccine. This may have been due to a lack of induction of CTL activity, while also, an unbalanced response against the viral F protein may have contributed to the occasional failure of this type of vaccine (110). Secondly, the levels of virus-neutralizing antibodies induced by inactivated vaccines have been shown to drop rapidly relative to antibody titers elicited by a natural measles virus infection (110). In this respect, it is promising that the liposome-supplemented inactivated measles virus antigen induces antibody responses that persist for at least 6 months after i.n. administration to mice (Chapter 5).

Dissemination of the s-IgA response to distant mucosa

In Chapter 6, it is shown that liposomes administered to the lower respiratory tract of mice have the capacity to induce an s-IgA in the female urogenital tract. This indicates that the s-IgA response, induced in the respiratory tract, disseminates throughout the common mucosal immune system. This phenomenon is probably a result of migration of IgA-committed B cells from the lymphoid tissue associated with the lungs to distant mucosal effector sites, such as the mucosa of the urogenital tract (19, 87). At these distant effector sites, the locally produced s-IgA antibodies will be actively transported into the mucosal secretions (90). Therefore, in addition to GALT, also the lymphoid structures associated with the lung can act as IgA-inductive sites. This finding is of interest for the development of vaccines that aim at the induction of a s-IgA response in the urogenital tract where this response may be beneficial in the prevention of, for example, sexually transmitted diseases.
Immune stimulation by liposomes

In Chapter 2 it is shown that local administration of a liposome-supplemented influenza virus subunit antigen results in high levels of systemic as well as local antibody production. In general, the immunostimulatory activity of liposomes has been attributed to an increased uptake of liposome-associated antigen by macrophages, e.g. alveolar macrophages (AM), thereby facilitating the presentation of antigen-derived peptides to T helper cells (7, 29, 52, 146). Liposomes have also been proposed to serve as a depot for slow release of antigen (42). Although these mechanisms may well be involved in the immunoadjuvant activity of parenterally administered liposomes, it is unlikely that these mechanisms play a major role in the mucosal immunoadjuvant activity of liposomes administered to the respiratory tract. Our studies demonstrate that, under these conditions, liposomes exert an additional immunoadjuvant activity that is independent of physical association of the antigen with the liposomes: immune stimulation is observed when liposomes are simply mixed with antigen or even when the liposomes are given up to 48 h prior to administration of the antigen (Chapter 2). In these cases, clearly, the liposomes do not act as an antigen-carrier system nor do they function as a depot for slow release of the antigen. Since the adjuvant activity of liposomes becomes apparent upon local administration to the lower respiratory tract, including the lungs (Chapter 2, 5 and 6), we conclude that the stimulated immune responses are initiated in the lymphoid tissue associated with the trachea and lungs. Here, AM, principal scavenger cells of the lungs, take up large quantities of the liposomes (Chapter 7). Studies by Holt et al. have shown that AM constitutively suppress immune responses in the lungs (67). The investigators demonstrated that AM suppress T cell responses and inhibit antigen presentation by resident pulmonary dendritic cells (68, 70). It was noted that the degree of inhibition was markedly increased by the presence of tumor necrosis factor α (TNF-α, ref. 70). Interestingly, AM-mediated suppression of antigen presentation by DC is abrogated by inhibition of the nitric oxide (NO) synthetase pathway (70). This suggests that the release of NO by AM contributes to suppression of pulmonary DC function. In accordance with the above, Thepen et al. have observed a stimulation of the formation of antigen-specific antibody-forming cells in the lungs of mice which were previously depleted of their AM population (139). Therefore, we hypothesize that uptake of liposomes by AM inhibits the AM-dependent immune suppression temporarily, thereby stimulating antibody responses against coadministered antigens. In support of this notion, we found that functional depletion of AM induces an enhanced systemic and local antibody response against influenza virus subunit antigen subsequently administered to the lower respiratory tract. This suggests that blocking of AM by uptake of liposomes mediates stimulated antibody responses, particular upon immunization of the
lung. Furthermore, the fact that negatively charged liposomes, but not zwitterionic or positively charged liposomes, stimulate systemic and local antibody responses against coadministered influenza subunit antigen (Chapter 2 and 3), may reflect the relative efficient uptake of negatively charged liposomes by AM (Chapter 7). In addition, it is interesting to note that, recently, Phillips and Gagne reported that negatively charged liposomes, but not zwitterionic liposomes, have the capacity to modulate the synthesis of NO in cultured murine macrophages (116). This observation suggests that negatively charged liposomes may interfere with NO-mediated suppression of DC function by macrophages, as discussed above. Alternatively, blocking by liposomes could inhibit the scavenger function of AM and rescue coadministered antigen from uptake and degradation. As a consequence, more antigen would be available for other cells better capable of antigen presentation, such as DC operating in concert with resident IM (50). Finally, we cannot formally exclude the possibility that uptake of liposomes may activate AM. It would be of interest to investigate whether uptake of liposomes upregulates MHC class II expression by AM. This could indicate an enhanced ability of the AM to present antigens leading to increased antibody responses. In addition, the assessment of cytokine secretion in the bronchoalveolar space could further indicate AM activation.

In conclusion, this thesis presents a study on the immunostimulatory activity of liposomes administered to the respiratory tract of mice. Liposomes are shown to stimulate systemic and local antibody responses against influenza subunit antigen and inactivated whole measles virus independent of physical association of the antigens with the liposomes. In addition to systemic antibody responses, the induction of mucosal antibody production is demonstrated. This response is not restricted to the site of antigen application, but disseminates to distant mucosal sites, such as the mucosa of the urogenital tract. The i.n. liposomal adjuvant provides a versatile adjuvant system for use in a variety of vaccines, particular in cases where induction of mucosal immunity is important.