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Priming or Tolerization of Tumor-specific T cell Immunity; Lessons from Murine Tumor Models

Most tumor cells show a disturbed pattern of gene expression. As a result, these cells exhibit qualitative and/or quantitative differences as compared to normal somatic cells with respect to the repertoire of MHC-bound peptides. The immune system is, in principle, able to react against such peptide/MHC complexes and, therefore, to the tumor cells bearing these antigens.

However, in spite of the expression of these antigens, most tumor cells are not able to directly prime T cells. This is because they cannot give a second, crucial signal to the T cells in addition to the antigenic stimulus: a costimulatory signal. Such costimulatory signals can be provided by professional antigen presenting cells (APC), such as dendritic cells (DC).

These cells are especially recruited to areas of the body which are disturbed, for instance as a result of invading pathogens. When they encounter such an area, they can take up antigens, such as derived from (tumor) cell debris.

These antigens are processed and the resulting peptides are presented by MHC molecules at the surface of the APC. Since such professional APC express both class I MHC and class II MHC molecules, they are capable of activating both class I MHC-restricted cytotoxic T lymphocytes (CTL) and class II MHC-restricted T-helper (Th) cells. Both types of T cells are in generally needed for a persistent and effective immune response. However, in order to be able of activating both arms of the T cell response, the APC also needs to give out costimulatory signals to the T cells, such as through the surface molecules B7.1/B7.2 and through interleukin-12. Importantly, the APC is only capable of providing these signals once it has been fully activated. This full activation requires it to receive certain triggers from its environment. Although the nature of these signals is only beginning to emerge, such >danger= signals are thought to require inflammation. If all requirements are fulfilled, the antigen-loaded APC can >cross-prime= a tumor specific T cell response.

Unfortunately, in many cases where tumors start to grow, one or more of the criteria for priming of the T cell response is not met. This can be caused by a lack of sufficient tumor antigens, but also because of the fact that tumor cell growth can proceed rather silently, especially at earlier stages of tumorigenesis. Since the local environment of a newly arising tumor does not necessarily become inflamed, mobilization

and activation of professional APC, and therefore T cell priming, will not take place. Moreover, various tumor cells have been found to secrete lymphokines, such as interleukin-10, which are known to counteract activation of APC. Failure of the APC-mediated cross-priming causes the immune system to be either ignorant of — or tolerant to — the tumor.

Tumor-specific vaccination aims at preventing or breaking this ignorance or tolerance. By exposing the immune system to significant amounts of tumor-associated antigens, the capacity of T cells to track down and eradicate tumor cells expressing these antigens can be increased. The first indications for the existence of tumor-specific immunity were obtained from experiments showing that animals which had succeeded in rejecting a tumor, or which had been injected with irradiated tumor cells, were resistent against a subsequent challenge with the same tumor cells. Although tumor cell-based vaccines were shown to induce effective anti-tumor immunity in many experimental tumor models, and have even been used in a clinical setting (e.g. (1-4)), the discovery of the first MHC-bound tumor-specific peptide-epitopes boosted the experimentation with more defined anti-tumor vaccines. Amongst the first tumor-specific T cell epitopes identified in murine tumor models were several peptides derived from viral onco-proteins. For instance, human adenovirus type 5 (Ad5) induced tumors of C57BL/6 (B6) origin were shown to present at least two highly immunogenic peptides to the T cell immune system. These peptides, which are derived from the Ad5 E1A and Ad5 E1B oncoprotein and both bind to the H-2D^b molecule, constitute targets of an effective cytotoxic T lymphocyte (CTL) response, as CTL against these peptides were shown to efficiently eradicate Ad5-expressing tumor cells in vitro as well as in vivo (5, 6). Similarly, human papilloma virus type 16 (HPV16) induced tumors of B6 origin were shown to present at their surface an H-2D^b-bound peptide epitope derived from the HPV16 E7 oncoprotein. Immunization of B6 mice with synthetic E7 peptide emulsified in incomplete Freund's adjuvant (IFA) was shown to induce strong peptide-specific CTL immunity. These CTL were capable of lysing HPV16-transformed cells in vitro and, more importantly, of protecting vaccinated mice against a subsequent challenge with an otherwise tumorigenic dose of these tumor cells (7).

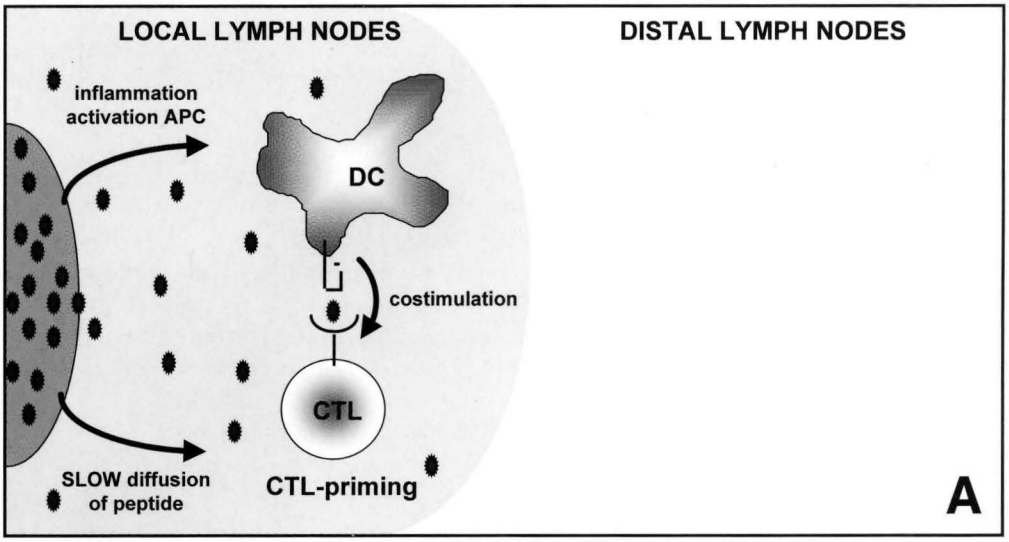
While this study was accompanied by various other reports demonstrating that immunization of mice with vaccines consisting of minimal CTL epitopes in IFA could induce protective pathogen- or tumor-specific T cell immunity (e.g. (8-11)), similar immunization experiments in the Ad5 tumor model rendered quite contrasting results. Vaccination of B6 mice with either the Ad5 E1A or E1B peptide in IFA was shown to result in enhanced rather than decreased outgrowth of Ad5-transformed tumors in these mice. This was a consequence of the specific repression of the peptide- and tumor-specific CTL response. Such adverse effects of peptide vaccination were found for both the E1A and E1B peptides, whereas comparable vaccine formulations comprising other peptides, such as derived from murine leukemia virus-induced tumors, HPV16 and Sendai virus, induced protective CTL immunity (12, 13). Of the various possible parameters that could account for the opposite in vivo effects of these peptide-epitopes, only one was found to show striking correlation with the

tolerizing capacity of the Ad5 peptides: the E1A and E1B peptides when injected subcutaneously in a depot of IFA were found to rapidly become dispersed throughout the animal (12). Such a rapid diffusion was not found for the HPV16 E7 peptide. The IFA-component of the vaccine has basically two functions: to serve as a depot from which the peptide gradually leaks out into the environment and to induce inflammation in the direct vincinity of the injection site. As a result of these two functions, the HPV16 E7 peptide will be loaded onto the class I MHC of various professional and non-professional antigen presenting cells, the former of which will be activated due to the IFA-induced inflammation. The end result is presentation of the epitopes in a proper costimulatory context which can lead to efficient CTL priming (figure 1A). On the other hand, when the immunogenic peptides become distributed systemically one could envision a situation as depicted in figure 1B. Due to systemic distribution, the peptide epitope will not only become presented by fully activated APC in the vincinity of the IFA-depot, but also by (amongst others) non-activated professional APC at distal sites which have not received inflammatory signals from the adjuvant. Since there are indications that presentation of T cell epitopes by especially non-activated professional APC will induce tolerance rather than T cell priming (14-16), it is conceivable that the net result of this situation will be peptide-specific T celltolerance.

Although this model still needs to be confirmed by further experimentation, the search for alternative, immunostimulatory vaccine formulations based on the tolerogenic Ad5 peptides has provided support for this notion. In vitro cultured and activated DC, derived either from bone marrow cells or from splenic monocytes, when loaded with either of the Ad5 peptides, were shown to induce strong protective anti-tumor CTL immunity. On the other hand, when these peptides were loaded onto normal bone marrow cells no CTL priming was observed (17). Therefore, the outcome of vaccination is not so much dictated by the Ad5 peptides per se, but rather by the context in which these peptides are presented to the T cell immune system. If these peptides are placed into an appropriate costimulatory context, such as on activated DC, efficient priming of the CTL response is achieved. Taken together, these data show that care should be taken with respect to the application of vaccines consisting of free minimal peptide epitopes emulsified in adjuvant, whereas activated DC constitute a formidable component of such vaccines.

In our search for alternative vaccine formulations comprising the potentially tolerogenic Ad5 peptide epitopes, we also found that recombinant adenoviruses encoding string-of-beads arrangements of minimal peptide epitopes were capable of inducing strong CTL immunity. One of the viruses tested carried a synthetic minigene encoding four tumor-associated minimal peptide epitopes, each spaced by a triple alanine sequence (figure 2).

Immunization of mice with this virus, encoding amongst others the Ad5 E1A, Ad5 E1B and HPV16 E7 epitopes, induced protective CTL immunity against both HPV16- and Ad5-induced tumors (18). The efficiency by which this virus can directly infect professional APC, such as DC, in vivo is likely to be low. It is therefore conceivable that vaccination results in infection of mostly other somatic cells, after which antigens derived from these cells are taken up, processed and presented by professional



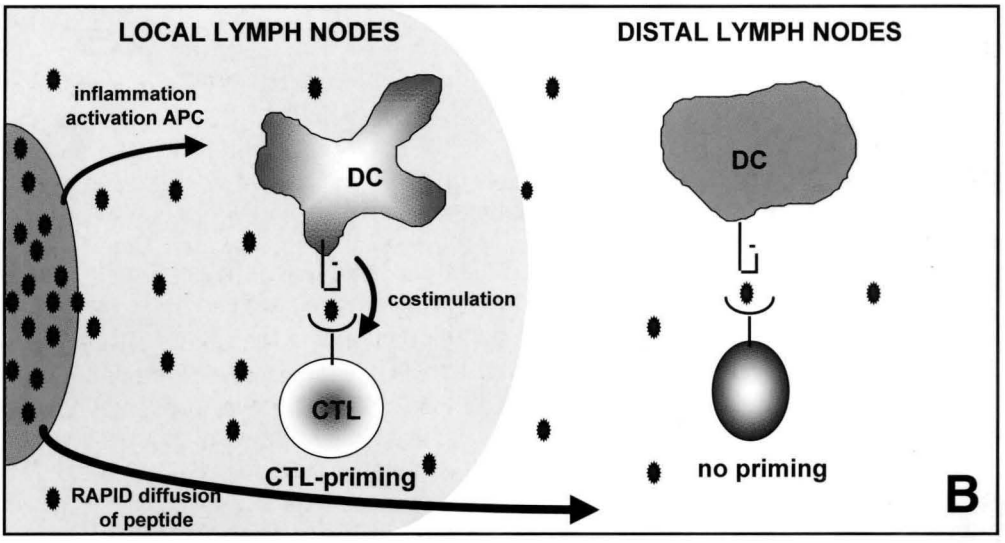


Fig. 1. Model offering a possible explanation for the fact that local distribution of the HPV16 E7 peptide leads to induction of CTL immunity (panel A), whereas systemic distribution of the Ad5 peptides results in specific T cell tolerance (panel B).

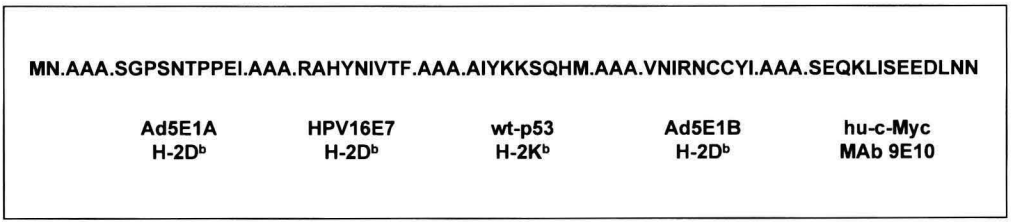


Fig. 2. Recombinant adenovirus vaccine carrying a synthetic minigene encoding a string-of-beads arrangements of CTL epitopes. The epitopes are derived from the Ad5E1A, Ad5E1B and HPV16 E7 oncoproteins. The fourth CTL epitope, not discussed in this review, is derived from the wild type murine p53 sequence (35). The C-terminal sequence, which is derived from the human c-Myc protein, is a recognition site for a monoclonal Ab (18).

APC that have been attracted by the inflammation caused by the high dose (10^8-10^9) of virus particles. Consequently, the peptide epitopes become presented in an appropriate costimulatory context and priming of protective CTL occurs.

As mentioned in the first paragraph, vaccination with (irradiated) tumor cells can induce tumor-specific T cell immunity. It is crucial that the tumor cells employed for vaccination express the relevant tumor antigens, whereas surface-expression of the relevant MHC/peptide complexes is usually not essential. For instance, vaccination of B6 mice with Ad5-transformed cells of either syngeneic or completely allogeneic (Balb/c) origin results in induction of equally effective Ad5-specific CTL immunity (19). Especially in the case of immunization with allogeneic tumor cells, the induction of T cell immunity is completely dependent on the action of host-derived professional APC, as the allogeneic cells themselves do no express the proper MHC molecules.

Since experimental data by us (see above) and others (e.g. (3, 20)) demonstrate the pivotal importance of cross-priming APC in T cell priming, we investigated this process in more detail by employing the allogeneic tumor cell vaccination setup described above. When such immunizations are performed in mice that have been depleted of $CD4^+$ T cells, or in class II MHC knock-out (ko) mice, no Ad5-specific CTL immunity is induced. This indicates that cross-priming of CTL immunity, at least in this setting, depends on the action of $CD4^+$ Th cells. A more classical view on T cell help is that these cells provide CTL with costimulatory signals mainly through lymphokines such as interleukin-2 (21, 22). More recent data however suggest that CD4 Th cells may also contribute to CTL priming through modulation of the host APC function (23). Since activated Th cells are known to express CD40-ligand (CD40L), whereas professional APC such as DC are know to express the corresponding receptor CD40 and to become fully activated upon triggering through this receptor (24-30), we focused on the interaction between Th cells and APC through the CD40L-CD40-pair.

Important tools were two antibodies (Ab): the activating anti-CD40 Ab FGK45 (31) and the anti-CD40L blocking Ab MR1 (32). Interestingly, when CD4-depleted mice or class II MHC ko mice were immunized with allogeneic Ad5-transformed

cells in combination with a dose of activating anti-CD40 Ab, the otherwise absent anti-Ad5 CTL response was completely restored.

Moreover, induction of this response in normal B6 mice could be abrogated by administration of anti-CD40L blocking Ab, while co-administration of activating anti-CD40 Ab again restored CTL priming. Comparable results were obtained in B cell deficient mice, indicating that CTL priming required triggering of professional APC, not of the B cells which also express CD40 (33). These data demonstrate the crucial role of CD40-mediated triggering of professional APC in T cell priming. Since this signal can be delivered by $CD40L^+$ Th cells, our data implicate that an important function of $CD4^+$ Th cells in CTL priming is to deliver an activating signal to the APC through CD40, which will result in presentation of T cell epitopes on the APC in a proper costimulatory context (figure 3).

In the experiments described above the activating anti-CD40 Ab was administered intraperitoneally, resulting in a systemic distribution of these Ab. It is therefore conceivable that the Ab will cause systemic activation of professional APC. This concept inspired us to combine the tolerogenic vaccine consisting of Ad5 peptides in IFA with administration of activating anti-CD40 Ab. Since we found this vaccination protocol to result in systemic distribution and presentation of the Ad5 peptides (12), whereas the anti-CD40 Ab will cause systemic activation of professional APC, one might expect this combination to cause CTL priming rather than tolerisation.

Indeed, preliminary data show that this combination treatment results in CTL priming for both the Ad5 E1A and the Ad5 E1B peptide (manuscript in preparation). This result further stresses the importance of proper management of the antigen presenting context in vaccine-development and indicates that agents capable of triggering CD40, such as activating antibodies or the recently described CD40L-trimer (34), may constitute promising components of immunostimulatory vaccines.

In conclusion, the identification of increasing numbers of MHC-restricted peptide epitopes has initially focussed the attention of many vaccinologists on the antigenic contents of vaccines. New insights in the mechanisms of antigen presentation and T cell priming however, indicate that provision of an appropriate costimulatory context is equally essential to the efficacy of vaccines. Failure to provide such a context can even result in tolerisation rather than T cell priming. Very strong costimulatory signals may be especially important in settings where the immune system is tolerized to the antigens of choice, such as may be the case for tumor-associated auto-antigens and in tumor-bearing subjects.

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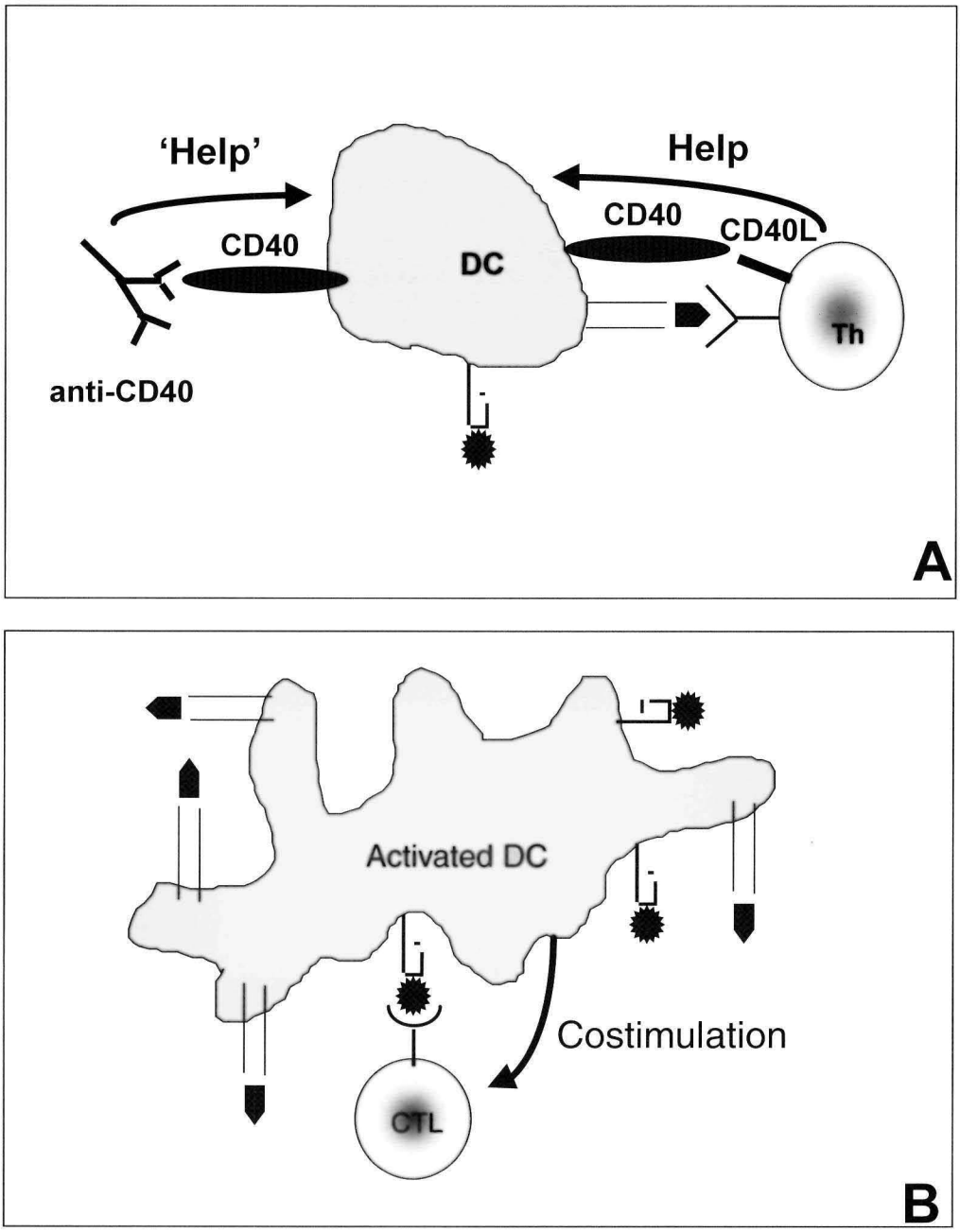

Fig. 3. CTL priming requires prior activation of professional APC. Dendritic cells (DC) can receive an activating signal through their CD40 receptor (panel A), after which they show strong upregulation of surface MHC expression, costimulatory molecules such as B7.1/B7.2 and interleukin-12 secretion. The combination of high antigen-density and strong costimulatory capacity makes DC very potent stimulators of CTL immunity (panel B).

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