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Review

Tumors and the danger model★

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This article reviews the evidence for the danger model in the context of immune response to tumors and the insufficiency of the immune system to eliminate tumor growth.

Despite their potential immunogenicity tumors do not induce significant immune responses which could destroy malignant cells. According to the danger model, the immune surveillance system fails to detect tumor antigens because transformed cells do not send any danger signals which could activate dendritic cells and initiate an immune response. Instead, tumor cells or antigen presenting cells turn off the responding T cells and induce tolerance.

The studies reviewed herein based on model tumor antigens, recombinant viral vectors and detection of tumor specific T cells by MHC/peptide tetramers underscore the critical role of tumor antigen presentation and the context in which it occurs. They indicate that antigen presentation only by activated but not by cancer or resting dendritic cells is necessary for the induction of immune responses to tumor antigens. It becomes apparent that the inability of dendritic cells to become activated provides a biological niche for tumor escape from immune destruction and seems to be a principal mechanism for the failure of tumor immune surveillance.

INTRODUCTION TO THE DANGER MODEL

In 1994 Polly Matzinger presented a new theory called the danger model suggesting that specific immune response develops as a result of danger detection rather than discrimination between self and non-self antigens (Matzinger, 1994). Although this model refers mainly to immune tolerance its as-

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Abbreviations: APC, antigen presenting cells; HSP, heat-shock proteins; MHC, major histocompatibility complex; TCR, T-cell receptor.

sumptions are valid for such distant subjects as tumors or apparently unrelated with immunology gene therapy.

The contemporary self-non-self discrimination model assumes that immune system protects the organism against everything which is foreign, thus immune responses are directed towards external entities which are non-self antigens (Janeway, 1992). According to the danger model the immune system detects and then responds to anything dangerous and not necessarily foreign (Matzinger, 1994; 1998; Fuchs & Matzinger, 1996). The whole model is based on the principle that the presence or absence of the so called second signal determines immune responsiveness or tolerance. Whereas the first signal comes from specific antigen recognition, the second signal is generated from either help delivered by T-helper lymphocytes or co-stimulation from professional antigen presenting cells (Ridge *et al.,* 1998; Schoenberger *et al.,* 1998; Bennett *et al.,* 1998). The outcome of antigen recognition (immune response *versus* tolerance) depends also on the differential status of the responding cells. The general rules to generate an immune response or, tolerant state for different cells are as follow (Matzinger, 1994):

A. Lymphocytes

I. Naive T cells

- 1. Undergo apoptosis if receive signal one in the absence of signal two
- 2. Second signal may be offered only by professional antigen presenting cells (now it is generally accepted that these are dendritic cells only)

II. Memory T cells

- 1. Undergo apoptosis if receive signal one in the absence of signal two
- 2. Second signal may be offered from B cells, macrophages or dendritic cells

III. B cells

- 1. Undergo apoptosis if receive signal one in the absence of signal two
- 2. Second signal may be offered only from memory/effector T cells

IV. Effector T and B cells

- 1. Perform functions after antigen recognition regardless of the presence or absence of signal two
- 2. Undergo apoptosis or revert to a resting state after a reasonably short period of time

B. Antigen presenting cells

I. Professional APC (i.e. dendritic cells)

- 1. Capture antigens from environment, go to draining lymph nodes and present antigens to T cells
- 2. In the presence of tissue destruction (i.e. damage) become activated
- 3. Express co-stimulatory signals that can be received by naive and memory T cells
- 4. Upregulate co-stimulatory molecules upon receiving proper signals from T-helper cells

II. B cells

- 1. Expressed co-stimulatory signals are for memory T cells only
- 2. Capture specific antigens, concentrate them and present to T cells.

Although the rules presented above are oversimplified, nevertheless they describe in a very clear and comprehensive way how immune response is induced and at the same time how tolerance can be maintained.

The main difference between the proposed danger model and the self-non-self discrimination theory is the way of the initiation of an immune response. The self-non-self discrimination theory assumes that being foreign is good enough to induce adaptive response (Janeway, 1992). In the danger model it is assumed that the main determinant leading to the initiation of an immune response is the presence of an antigen in the context of tissue destruction. If there is no damage and cells are unharmed or they die by apoptosis no immune response ensues. However, if cells are injured, stressed or die by necrosis an immune response is induced (Matzinger, 1994; 1998; Fuchs & Matzinger, 1996).

Since an absolute majority of body cells present only signal one, the default reaction of a

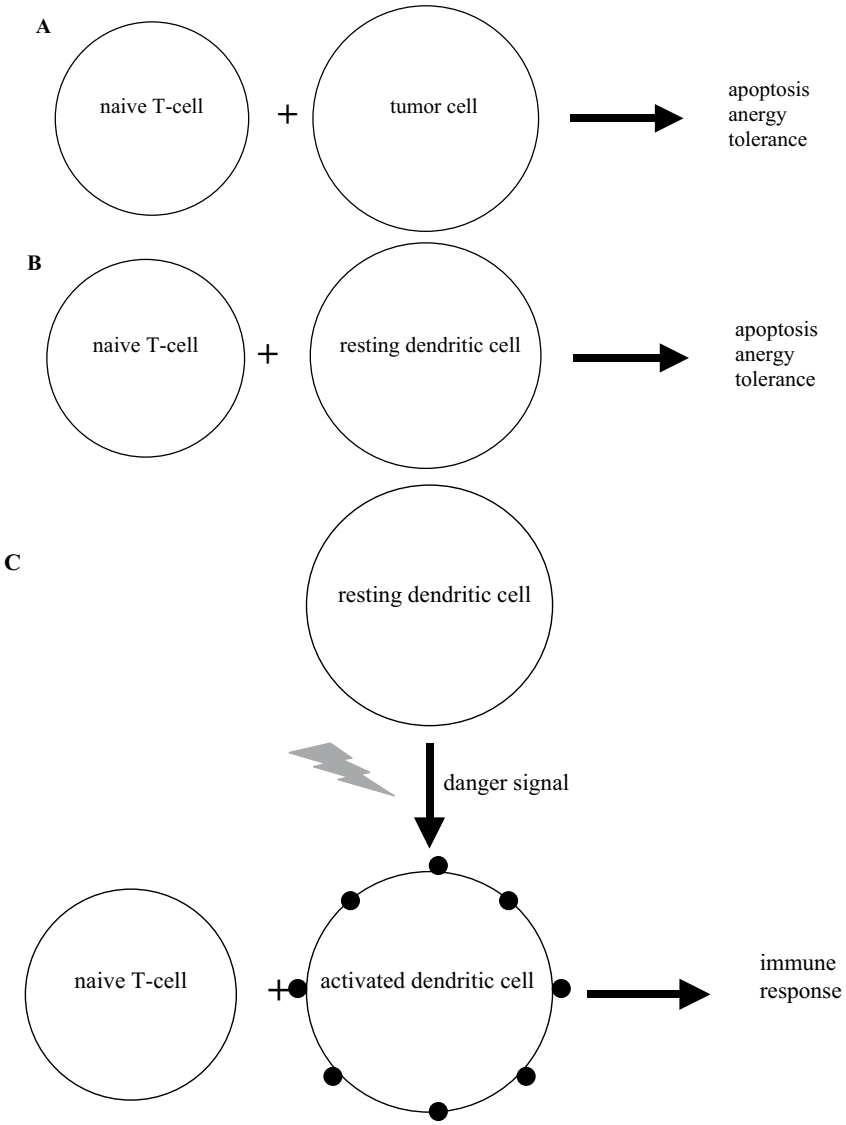

Figure 1. Co-stimulation is required for activation of naive T cells and induction of an immune response.

Tumor antigen recognition by a naive T cell directly on tumor cells (A) or resting dendritic cells (B) due to a lack of co-stimulation leads to tolerance instead of induction of an immune response. "Danger signal" induces co-stimulatory molecules such as CD80 and CD86 (●) on dendritic cells which provide a second signal for T-cell activation and make them capable of induction of primary immune response (C).

responding T cell for an antigen should be tolerance. This kind of behavior is very beneficial to sustain the tolerant state and to avoid auto-aggression against self tissues. In order to initiate an immune response, antigen presenting cells have to detect all suspicious conditions connected to tissue damage and inform T cells. Tissue injury due to any pathological process should thus lead to dendritic cell activation, which loaded with surrounding antigens and armed in co-stimulatory molecules go to draining lymph nodes. Such activated dendritic cells are thus ready to present the captured antigens and induce an immune response.

MOLECULES INDICATING DANGER

The danger model proposes that dendritic cells cannot constitutively deliver the co-stimulatory signals. Instead, they have to be activated only when needed in situations of danger. In order to be activated in such a situ-

ation antigen presenting cells must have some kind of receptors which could see and recognize any signs of tissue distress. In the simplest scenario dendritic cells might be activated directly by some viruses, viral proteins or even foreign bacterial DNA (Wu & Liu, 1994; Xiang *et al.,* 1996; Lenz *et al.,* 2001; Ulmer *et al.,* 1993; Kowalczyk & Ertl, 1999). It has been shown that adenoviruses or purified DNA may transduce and activate (i.e. induce expression of co-stimulatory molecules) dendritic cells (Wu & Liu, 1994; Xiang *et al.,* 1996; Lenz *et al.,* 2001; Ulmer *et al.,* 1993; Kowalczyk & Ertl, 1999). This feature makes recombinant adenoviruses very suitable as vaccines carriers (Xiang *et al.,* 1996). Similarly to the whole viruses, purified viral proteins may do the job as well (Lenz *et al.,* 2001). The danger signal might be also delivered from neighboring cells which send it upon stress, such as infection, hypoxia, trauma, etc. Although they have not been completely identified, some molecules are very likely to be good candidates for such mediators. Heat shock proteins (HSP), synthesized by cells in response to a variety of stressors, seem to be a prime "suspect" for the danger signal. Indeed, Srivastava and co-workers have shown that HSP play a critical role in cross-priming (Suto & Srivastava, 1995; Udono *et al.,* 1994), an immunologic phenomenon whereby antigens expressed by one cell are presented by dendritic cells to induce an immune response. Recently they have demonstrated that dendritic cells express specific receptors for such proteins (Binder *et al.,* 2000; Basu *et al.,* 2001). Some other danger signals that recently have been described are nucleotides, reactive oxygen intermediators, and cytokines such as interferons (Galluci & Matzinger, 2001**).**

EXPERIMENTAL EVIDENCE

The phenomenon that in certain circumstances completely foreign proteins do not induce an immune response is well known among immunologists (Matzinger, 1994). Moreover, in order to display good immunity additional substances called adjuvants, which in a non specific way augment the response, must be used. According to the danger model these additional, usually irritating, materials deliver or induce the required danger signal.

In a gene therapy model for type B hemophilia the type and magnitude of the immune responses against a transgene were strictly depended on vectors used for *in vivo* gene delivery. Intramuscular injection of an adeno-associated vector (AAV) expressing factor IX failed to activate factor IX-specific cytotoxic T lymphocytes (CTLs) in hemostatically normal or in hemophilia B mice, thus indicating an absence of cellular immune responses against factor IX. The same gene delivered by recombinant adenoviruses efficiently induced a strong, factor IX-specific cytotoxic T cell and T-helper cell immune response, leading to inflammation, destruction of transduced muscule tissue and activation of B cells (Fields *et al.,* 2000).

A similar lack of immunogenicity of foreign antigens has been observed in several studies with model tumor antigens. Although immune responses to tumor-associated antigens provide a substantially different biological context, similar questions (i.e. if and how tumors can induce an immune response) have been asked. Potential mechanisms of T-cell activation by tumor cells are simplified in that endogenous production of tumor-associated antigens in professional APCs is not possible.

The most informative results have emerged from experiments based on direct enumeration of tumor-specific T cells. One of the most powerful new tools are fluorescently labeled recombinant tetramers of MHC class I molecules containing a nominal antigenic peptide to stain specific CD8 T cells (Altman *et al.,* 1996). The technique is based on multimers of the natural ligand for the T-cell receptor, the peptide–MHC complex, with sufficient affinity for the TCR to permit their use as staining reagents in flow cytometry. Other sensitive

new techniques involve specific T-cell stimulation with antigenic peptides or whole, live tumor cells followed by measuring cytokine production at the single-cell level by intracellular staining (Kowalczyk *et al.,* 2000). These approaches allow direct visualization of antigen specific (i.e. tumor specific) CD8 T cells, and the analysis can be done on freshly explanted cells without any *in vitro* manipulations.

Tumors often induce extensive T cell infiltration *in vivo*, but the specificity of the responding T cells has not been defined. To address this issue we used tetramers of MHC class I molecules containing immunodominant model tumor antigen peptides to directly visualize tumor-specific CD8 T cells during tumor development in mice (He *et al.,* 2000; Kowalczyk *et al.,* 2001). Studies based on tetramer binding and a sensitive assay measuring interferon-gamma production at the single-cell level, in mice challenged with tumor cells expressing the E6 and E7 human papilloma virus (HPV) oncoproteins, have shown that the tumor bearing animals did not develop any assessable immune response measured at the tumor site, in spleen, lymph nodes or peripheral blood (He *et al.,* 2000; Kowalczyk *et al.,* 2001). This lack of the immune response to these foreign antigens was due to the complete absence of antigen specific T cells and not to their anergy. Direct staining with MHC/peptide tetramers did not detect any E7 peptide specific T cells. Similarly, *in vitro* stimulation with the peptide did not induce any interferon gamma production. It should be noted that cells expressing E6 and E7 can be recognized and killed by specific, sensitized CD8+ T cells (Kowalczyk *et al.,* 2000; He *et al.,* 2000; Kowalczyk *et al.,* 2001). However, immunization with recombinant adenovirus encoding the E7 oncoprotein followed by challenge with live cells expressing the E7 oncoprotein increases the number of antigen (i.e. E7) specific T cells *in vivo* and leads to complete protection against the tumor (Kowalczyk *et al.,* 2000; He *et al.,* 2000). Moreover, tumor regression was associated with very intense specific T cell infiltration providing strong support for the concept that immunosurveillance by anti-E7 $CD8^+$ T cells protects against tumors expressing the E7 antigen (Kowalczyk *et al.,* 2000). Thus, depending on preexisting conditions (naive *versus* immune) and the way the antigen is presented there will be no immune response or there might be even a booster effect.

Studies on different vectors for gene therapy or vaccination purposes have shown that the only cells responsible for the induction of immune response are the dendritic cells (Jooss *et al.,* 1998).

Thus, the antigen presented by macrophages or B cells will not induce a primary immune response (Fuchs & Matzinger, 1992). These studies stress the importance of dendritic cells as the cells solely responsible for antigen presentation to naive T cells and the induction of immunity.

TUMORS AND THE DANGER MODEL

It is thought that transformed tumor cells express antigens which are either completely absent or expressed in very small amounts on somatic cells. In general, there are four possible mechanisms that may lead to the appearance of these antigens: mutation, virus infection, gene activation and clonal amplification. Proteins encoded by mutated genes, viral antigens or unique clonal structures such as the idiotype of surface immunoglobulin B-cell malignances are all potential targets which could be recognized by the immune system as foreign. Thus, in most cases tumor cells expressing such antigens should easily be eliminated. Unfortunately, it is not as easy as one might expect. Tumor cells can escape or fail to elicit tumor specific immune responses by various mechanisms. It is postulated that transformed cells are genetically and phentotypically less stable than normal cells and can rapidly adapt to new conditions and escape immune destruction. The ways tumors may

become unrecognizable to the immune system are numerous. Although tumor cells may express mutant proteins, they may lack mutant peptides that can be presented by the MHC molecules, they may lack MHC class I molecules or may have deficient antigen processing. Whereas deficient antigen processing or no MHC expression on tumor cells explain why cancer cells can not be recognized and than destroyed by antigen specific T cells, it does not explain why it is so difficult to find significant numbers of tumor reactive T cells in tumor bearing hosts. Since the immune response is initiated *via* antigen presenting cells during the cross-priming process antigen presentation on tumor cells is not absolutely necessary in the priming phase. Thus, it should be possible to see specific T cells against tumor antigens. These T cells might not necessarily respond to autologous tumors but they should be detected in a relatively easy way. As we know from everyday practice this is not the truth. The lack of tumor specific T cells might be explained in such cases by negative factors that influence overall immune responsiveness. Indeed, many immune defects during tumor progression have been described and cancer patients often display weakened immune response. However, laboratory animals usually display full immune competence but still do not respond to implanted tumors (Kowalczyk *et al.,* 2001). It seems that there must be a single, more general rule responsible for such a strange behavior of the immune system.

Similarly to other tissues, tumor cells do not express co-stimulatory molecules. Antigens presented by these cells will induce tolerance despite their complete foreignness. Assuming that transformed cancer cells do not do any damage in their surroundings (at least at the beginning when the tumor is not advanced) they are not recognized as dangerous and are ignored by the immune system. Tissue destruction or necrosis which could activate dendritic cells occurs latter in tumor development, usually after several years of an occult tumor growth. During that time tolerance toward tumor antigens is very likely to develop. Moreover, tumor necrosis is usually confined to their central parts and enclosed by "healthy" tumor cells which efficiently separate it from infiltrating dendritic cells (Bell *et al.,* 1999). Such a situation again is not optimal for the initiation of an immune response.

Large tumors possess another feature which makes them difficult to eradicate by the immune system. Even if there is an effective immune response evoked for example by a tumor vaccine, the danger model predicts that the vaccine induced response will eventually wane. First, as a result of the limited life span of effectors (see above, the rules) and later because there will be no further danger signals to maintain the response. It is thought that cytotoxic T cells kill tumors by inducing apoptosis which does not elicit alarm signals (however, there is growing evidence that it is not necessarily so), which could boost or keep up the immune response. Consequently any tumor cells left will grow not because they have escaped the immune systems but because the immune response stopped on its own (Fuchs & Matzinger, 1996).

CONCLUSIONS

Although many mechanisms of tumor escape from the immune surveillance have been described the danger model explains in a simple, unified and general way why there is no immune response to cancer cells despite foreignness of tumor antigens. Growing tumors do not provide a danger signal for dendritic cells and thus do not activate the immune system. Any tumor antigen-specific T cell will thus have its first antigen encounter with tumor cells or "resting" dendritic cell. Since there is no co-stimulation, either situation will drive the T cells into anergy or apoptosis and eventualy tumor tolerance (Staveley-O'Carroll *et al.,* 1998).

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