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Dendritic cells as vectors for immunotherapy.

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Dendritic cells (DC) constitute a unique system of cells able to induce primary immune responses. Distributed as sentinels throughout the body, DC are poised to capture antigen, migrate to draining lymphoid organs, and, after a process of maturation, select antigen-specific lymphocytes to which they present the processed antigen, thereby inducing immune responses.

DC represent both vectors and targets for immunological intervention in numerous diseases. Their unique ability to induce/enhance immune responses makes them an optimal candidate for vaccination protocols both in cancer and infectious diseases. Thus, DC loaded with appropriate tumor-derived or pathogen-derived antigens may *in vivo* produce protective/rejection immune responses leading to elimination of the disease. DC-mediated induction of immunity may be considered at three levels: 1) Ag loading and processing, 2) selection and activation of CD4⁺ T cells and 3) generation of effectors including antibodies and cytotoxic T cells. Each level raises specific questions that need to be addressed for the optimal use of DC in immunotherapy protocols.

Preliminary human trials in cancer patients reported up-to-date utilize DC isolated from blood (loaded with lymphoma idiotype) or *in vitro* differentiated from monocytes (loaded with melanoma or prostate cancer specific peptides). These approaches allow relatively easy generation (which occurs without concomitant proliferation) of homogenous DC populations. An alternative strategy to generate DC relies on the use of CD34⁺ HPC.

We are currently initiating clinical trials, in melanoma, prostate and breast cancer patients, which are designed to allow the comparison of the therapeutic efficiency and the extent of tumor-specific immune responses induced by: 1) Administration of different DC subpopulations, i.e. derived from proliferating CD34⁺ progenitors or from CD14⁺ blood precursors, 2) Administration of DC loaded with different sources of TAA, i.e. tumor antigen peptides or cellular bodies obtained from allogeneic tumor cell lines, 3) Combination therapy based on the injection of DC loaded with tumor cell bodies together with naive or primed T cells. We believe that the results of these studies will permit us to establish optimal conditions for successful DC immunotherapy as well as, eventually, allow the identification of immunodominant antigens.

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Induction of Anti-Tumor Immunity by Chemokine-ScFv Fusions in Murine B cell Lymphomas. Arya Biragyn^{1*} and Larry W. Kwak².

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Chemokine receptors are differentially expressed on APC, and it has been shown that immature and mature dendritic cells (DC) switch in chemokine receptor expression and are selectively recruited by distinct chemokines expressed in different anatomic sites. Here we present a novel approach utilizing these chemokine properties in order to produce effective lymphoma vaccine. Chemokine moieties were genetically fused to lymphoma derived idiotype, single chain antibody (sFv), a non-immunogenic self-tumor antigen, and tested as protein or naked DNA vaccine in two murine B cell lymphomas, 38C13 and A20. The chemokine-sFv fusion proteins bind respective chemokine receptors, elicit chemotactic responses *in vitro* and induce inflammatory responses *in vivo*. DNA vaccinations encoding corresponding chemokine fusions elicited significant protective anti-tumor response in both models tested. Moreover, naked DNA vaccinations with chemokine-sFv expressing constructs significantly delayed growth of an established tumor. Fusions with irrelevant sFv, sFv fusion with a truncated chemokine which lacked receptor binding and chemotactic activity, and mixtures of free chemokine and sFv proteins, or naked DNA plasmid vaccines encoding unlinked sFv and chemokine, failed to elicit immunity. The requirement for linkage of conformationally intact sFv and functionally active chemokine strongly suggested that the mechanism underlying these effects was the novel targeting of APC for chemokine receptor-mediated uptake of antigen, rather than the simple recruitment of APC to tumor by the chemokine. In addition to superior potency, the induction of critical effector T cells distinguished these fusions from other lymphoma Ig fusions. Chemokine fusion may provide a general strategy for eliciting T- cell immunity to other weakly immunogenic, clinically relevant antigens.

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Characterization of Tumor Vaccines During Product Development

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Tumor vaccines intended to generate immune responses manifested by local inflammation, delayed type hypersensitivity reaction, and tumor regression comprise a variety of products which can be grouped into one or more of the general areas: 1) cells, such as manipulated or nonmanipulated autologous or allogeneic tumor cells, activated peripheral blood or bone marrow-derived lymphocytes, dendritic cells or other antigen presenting cells; 2) immune regulatory agents, such as gangliosides and keyhole limpet hemocyanin; 3) antigen preparations, such as synthetic peptides, purified antigens, tumor cell lysate, a mixture of soluble tumor antigens mixed with adjuvants for injection or used *ex vivo* to activate various cell types; 4) gene modified cells, such as tumor cells or other cells engineered to secrete factors, cytokines, chemokines or surface expression of foreign major histocompatibility complex, co-stimulatory and immune regulatory molecules; 5) viral and plasmid vectors and liposome encapsulated plasmid vectors expressing cytokines, growth factors or tumor antigens. There are numerous clinical trials ongoing at various stages of tumor vaccine development. Some have advanced to pivotal or Phase III stages of clinical development. However, several major regulatory and scientific issues associated with clinical use of tumor vaccines have not been satisfactorily addressed. General regulatory principles are applied in the review of tumor vaccine products. These issues associated with different types of vaccines and general recommendation for the characterization of tumor vaccines at various stages of development will be discussed.

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CpG Immune Stimulatory Sequences For Therapeutic Vaccines

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Bacterial and vertebrate DNAs differ by the absence of CpG suppression or methylation in bacteria. The immune system has evolved to detect the presence of unmethylated CpG dinucleotides in particular sequence contexts ("CpG motifs") as a signal of infection. B cells are induced to proliferate and secrete immunoglobulin; dendritic cells secrete a wide array of cytokines, interferons, and chemokines and express increased costimulatory molecules. The cytokine response is dominated by IL-12, creating an environment that promotes Th1-like immune responses. CpG DNA costimulates B cell activation through cell membrane Ig thereby promoting the development of antigen-specific responses. Together, these factors activate a coordinated set of immune responses that include innate immunity (macrophages, monocytes, dendritic cells, and natural killer cells), humoral immunity, and cellular immunity. Effective genetic vaccination requires CpG motifs within the plasmid vectors. Immune stimulation by CpG DNA has potential prophylactic and therapeutic applications for vaccination against infectious and atopic diseases, as well as in cancer immunotherapy. CpG motifs combined with protein vaccines significantly improve the resulting immune response, and protect against challenge with pathogens or tumors. CpG motifs have antitumor activity in mice either when used as single agents to activate NK cells, or when combined with other immunotherapies. By virtue of its Th1-promoting effects, CpG can reverse certain experimental models of Th2-dependent diseases such as murine asthma.