CYTOKINE MODULATION OF IMMUNITY TO HUMAN PAPILLOMA VIRAL PROTEIN E7: EFFECTS OF THE LOCAL PRODUCTION OF CYTOKINES ON CTL DEVELOPMENT AND FUNCTION

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SYNOPSIS: We will utilize a gene transfection system to examine the generation of cellular immunity to tumor antigens in a mouse model. We will use the E7 oncogene of papillomavirus as a prototypic tumor antigen. The ultimate goal of such a system is to understand the underlying mechanisms involved in the generation of cytotoxic T lymphocytes (CTL) that are capable of eliminating tumor cells. We are concentrating on the generation of CTL since they can exhibit immunologic "memory", that is, a faster and stronger response upon re-exposure to a given antigen. Further, once generated, CTL circulate widely throughout an organism, and thus could eliminate metastatic tumor cells at a distant site. Using fibroblasts or cell lines transfected with cytokine genes and the E7 oncogene as a novel method for enhancing cellular immunity to tumors, we will specifically address how the local production of cytokines can regulate the primary generation of anti-tumor effector cells and establish a memory response.

BACKGROUND AND SIGNIFICANCE

The role of the immune system with respect to cancer has a long history. Indeed, the genetics of tumor transplantation between inbred strains of mice led to the discovery of the Major Histocompatibility Complex (MHC) of genes. We now know that the products of the MHC genes are not only the critical targets for graft rejection but also are essential for the normal functioning of the immune response. The class I gene products of the MHC (called the H-2K,D, L in mice and HLA-A,B,C in humans) associate with peptides during their biosynthesis and transport to the cell surface. The class I associated peptides are typically derived from proteins synthesized endogenously in the cytoplasm. Peptides derived from viral proteins are the classic example of foreign peptides that associate with class I. The immune cells which recognize this complex of class I/peptide are CD8 positive cytotoxic or killer T cells. In contrast, the class II molecules of the MHC are able to complex with peptides arising from proteins outside the cell or with peptides which are derived from proteins which are endogenously synthesized.This class II/peptide complex is recognized by CD4 positive helper T cells (1,2). These helper T cells produce cytokines which aid in triggering B cells to produce antibody and may help in the development of cytotoxic T cells. Cytokines have a variety of effects on the immune system, many of them affecting the proliferation and/or differentiation of immune cells (3,4). For example, the cytokine IL-2 is required for T cell growth (3,4). The role of other cytokines in the maturation of CTL is not as clear. In this proposal, we will investigate the role of cytokines in the generation of CTL using the product of an oncogene as a target antigen.

For many tumors there is evidence of an immune response in the host. However, frequently this response is a case of " too little, too late". The kinetic component of this response is particularly important in the case of tumors since one can envision a race between the tumor's ability to grow and the response of the immune system. It has been speculated that one of the main functions of the immune system is to eliminate neoplasms and that it is a failure of this function that results in the growth of tumors. This theory of "immune surveillance", while very appealing, remains controversial. However, whether or not this is the immune system's main role, it is becoming evident that the immune system can play a role in recognizing tumors. Perhaps a more critical issue is whether the immune system can be manipulated in such a way as to engender a potent anti-tumor response.

In this proposal we will focus on the specific cellular immune response to a human papilloma viral protein E7 from HPV16 as a model system for the genesis of CTL reactive with a defined tumor antigen. Human papillomaviruses (HPV) are a group of viruses associated with human cervical cancer (5,6). Further, the E7 protein is expressed in cervical carcinomas (7). E7 provides a potential target antigen for immune intervention. One of the major problems that has plagued tumor immunologists is the identification of specific tumor antigens. This has made it difficult to assess the specificity of the response. Thus one of the major advantages in the HPV system is that one has a defined "tumor antigen " to which a specific immune response can be measured. While many of the genes of HPV have been cloned and characterized we will concentrate on the E7 gene because it is known to be

involved in the transformation process and is not found on normal cells. Recent work has indicated that fibroblasts expressing E7 can generate a CTL response in mice (8). We will use this system to explore the effect that local production of cytokines has in regulating a specific immune response. Our hypothesis is that the local production of cytokines will dramatically modulate both the strength and type of immune response. The E7 transfected cells will provide a defined "tumor antigen" to assess how these cytokines affect a specific CTL response. Our two major aims are described briefly below.

Aim I: How do selected cytokines such as IL-2 affect the generation of cytotoxic or killer T cells specific for the E7 gene of human papillomavirus?

We will examine the effect of expressing an oncogene (in this case E7) which will serve as a target antigen and various cytokines on fibroblasts used for immunization. These experiments will examine the ability of fibroblasts which express cytokines to prime mice *in vivo* for an E7 specific response. In this aim we will examine the ability of selected cytokines including IL-1, IL-2, IL-3, IL-4, IL-6 and gamma interferon to enhance a specific immune response against the E7 gene product. These particular cytokines were chosen because of their known effects on T cell proliferation and their role in the generation of CTL (3,4).

We have already constructed vectors that drive the expression of several mouse cytokine genes including IL-2, IL-3, gamma interferon, and IL-6 using the high level expression vector system driven by the β-actin promoter. We have shown that all of these constructs are functional by transfection assays. Further, we have versions of the β-actin expression vector with different selectable markers (neo or gpt) which allows one to transfect and select for multiple genes in the same cell. We have also transfected and expressed the E7 gene in fibroblasts. Our recent data showed that we can generate a measurable CTL response to the E7 transfected fibroblasts. We hypothesize that effective immunity can be enhanced by increasing the concentrations of cytokines within the local environment of the transfected cells. In thefirst experiments we will examine the effect of IL-2 on the generation of E7 specific CTL. We have also successfully transfected fibroblasts with both the β-actin IL-2 plasmid and the E7 oncogene. These fibroblasts produce high levels of IL-2 (>200 units/ml) and also express the E7 antigen as demonstrated by immunoprecipitation. We will compare the ability of fibroblasts that have been transfected with E7, IL-2 and E7, or IL-2 alone to generate CTL which can kill E7 expressing cells. The efficacy of other cytokines will be examined in a similar fashion. Essentially, this will be a "memory" experiment in that we will assay spleen cells that have been primed *in vivo* and restimulated in vitro, for the presence of E7 specific CTL

Aim II: How does the local production of cytokine alter the early immune responses to the E7 oncogene?

The fibroblasts we described in the first aim do not make tumors in immunocompetent mice making it difficult to examine the early immune reactions. To assess the early immune responses to the E7, we will transfect the E7 oncogene into tumors (either Line 1 or EMT6) which have already been transfected with cytokines driven by the strong β actin promoter. We will then take advantage of our ability to isolate infiltrating lymphocytes from tumors. We will then analyze the cells that infiltrate using methods we have developed using magnetic beads to isolate Tumor Infiltrating Lymphocytes (TILs). These TILs, as well as the draining lymphoid organs, will be assayed functionally for their cytotoxicity and phenotyped by flow cytometry. Further, the tumors and the infiltrating cells will be characterized by classical histologic techniques and by immunocytochemical procedures. The advantage of this tumor system is that early immune responses can be analyzed. Thus one can assess within the tumor the type of effectors generated as well as their numbers. In addition, *in vivo* depletion studies with antibodies directed against CD4 or CD8 will help determine the mechanism of how the cytokines are enhancing immunity. We view these Aims as complementary ways of examining how cytokines can modulate an immune response to an oncogenic protein.

The current thinking is that the initial site of most immune responses is in lymphoid organs, such as lymph nodes, with the immunizing antigen either being trapped there or carried to this site via

antigen presenting cells such as macrophages. We hypothesize that one of the major roles of these lymphoid organs is to provide a local environment in which cells that have the antigen (antigen presenting cells) can be in close proximity to the T cells. This trigger consists of the MHC/peptide complex and the appropriate signals such as cytokines. Some of these cytokines such as IL-1 can be produced normally by the antigen presenting cells whereas others such as IL-2 are more typically produced by helper T cells. We anticipate that we can modify the fibroblasts or tumor cells using recombinant DNA techniques to enhance their ability to present antigen to T cells. In essence, this technique would bypass the lymph node and T helper cells by substituting the cytokines produced by the immunizing fibroblasts. While it is likely that other cells such as macrophages, mast cells, or granulocytes may be activated in the local environment of the cytokine transfected fibroblasts, we will focus on the generation of Cytotoxic T-Lymphocytes (CTL). These CTL will likely be the critical effectors in generating an effective memory response. Further, it is likely that CTL will be the most effective cell type at specifically attacking cells at distant sites which would be critical in eliminating metastases.

IMPACT OF RESEARCH

We believe the experiments outlined above will help determine the critical factors in generating a potent CTL response. The cellular immune response is critical for eliminating cells that are either infected with viruses or have become cancerous. The major type of cell for specifically eliminating these cells is cytotoxic or "killer" T cells. The objective of this study is to develop ways to improve the generation of tumor specific cytotoxic or "killer" T cells using genetically altered cells. We will use fibroblasts or established mouse tumor lines which have been altered so that they express a molecule found on tumor cells (the E7 gene from human papillomavirus) and cytokines which stimulate and regulate the immune system. In this system the E7 will serve as a model "tumor antigen" and allow study of specific immunity to a defined antigen that is only present on the cancer cells. These studies will lead to increased insight into the fundamental mechanisms by which CTL are generated. The P.I., John Frelinger, and the co-P.I., Edith Lord, bring complementary expertise (molecular biology/cellular immunology and tumor biology) to the project which will allow a multidisciplinary approach to understand how CTL are generated. We believe this represents a new and promising approach to examine the immunity to tumors using a defined tumor antigen. Further, such research has implications for vaccines in general that depend upon a CTL response.

BUDGET: We believe these goals can be accomplished in 3 years with an annual direct cost of $66,000.

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