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LUDWIG INSTITUTE FOR CANCER RESEARCH

LAUSANNE BRANCH

ANNUAL RESEARCH REPORT

2003

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BRANCH DIRECTOR'S REPORT

It is well established that cancer development in a patient is not solely dependent on the intrinsic properties of tumor cells but is the net result of complex interactions between tumor cells and a variety of normal cells within a given tissue. Among host-tumor interactions, lymphocyte-mediated activities are thought to play a major role in the control of tumor cell proliferation and dissemination. The aim of the work performed at the Lausanne Branch is to delineate the various mechanisms used by different lymphoid cell types to inhibit tumor cell growth. The current research activities are focused on the molecular and cellular mechanisms that regulate immunity mediated by T cells, natural killer (NK) cells and NKT cells, and include a strong patient-oriented program devoted to the development of therapeutic cancer vaccines.

In 2003, Dr. Hans Acha-Orbea, head of the Viral Immunology group, who had a joint appointment as Associate Professor in the Department of Biochemistry, University of Lausanne, since 1994, left the Branch to take up a full-time position in the same Department. We thank him for providing leadership and scientific excellence to the Branch for the past fifteen years and look forward to continuing our scientific collaborations with him and benefiting from his expertise in the context of the immunology training and research program carried out at the University of Lausanne. The links with the latter will be strengthened by the recent appointment of three of our group leaders, Dr. Pedro Romero, Dr. Frédéric Lévy and Dr. Freddy Radtke as Professor (P. R.) and Assistant Professors (F. L. and F. R.), respectively, in the newly created Faculty of Biology and Medicine.

In addition to its core research, the Branch is actively involved in the National Center for Competence in Research program entitled "Molecular Oncology : from basic research to therapeutic approaches", that has been established by the Swiss National Science Foundation and is carried out in partnership with the Swiss Institute for Experimental Cancer Research (ISREC) and the Multidisciplinary Oncology Center (CePO) at the Lausanne University Hospital. The NCCR program has opened up new collaborative links between scientists and clinicians in the area of translational cancer research and will serve as a model system for the implementation in Lausanne of an interdisciplinary consortium in cancer research in the near future.

J.-C. CEROTTINI

RESEARCH REPORT

CELL FATE DETERMINATION GROUP

The Cell Fate Determination Group led by Dr. Freddy Radtke is interested in the molecular mechanisms controlling stem cell maintenance, lineage commitment and differentiation in self-renewing systems such as the hematopoietic system, the skin and the gut. In addition this group investigates the potential role of these mechanisms in tumorigenesis.

Current attention is focused on the Notch signaling pathway, which is responsible for numerous binary cell fate decisions in diverse organisms. Previous studies by the group using conditional gene targeting strategy have established an essential role for Notch1 in specifying the T cell lineage. In addition a novel function for Notch1 has been uncovered at a later stage of T cell development, where it is involved in the control of VDJ rearrangement at the T cell receptor (TCR) beta locus. In the skin an unexpected function of Notch1 as a tumor suppressor gene has been discovered.

DEVELOPMENTAL IMMUNOLOGY GROUP

The Developmental Immunology Group led by Dr. Rob MacDonald continues to be interested in the development of T cells and in particular a subset of nonconventional T cells known as NKT cells. NKT cells utilize a semi-invariant $\alpha\beta$ TCR to recognize glycolipids presented by the monomorphic CD1d molecule. This semi-invariant TCR is comprised of an invariant $V\alpha 14-J\alpha 18$ chain paired preferentially with $V\beta 8.2$ and $V\beta 7$ chains. Using novel dimeric CD1d-IgG molecules complexed with the artificial agonist glycolipid α -galactosylceramide, the group has found that the $V\beta$ domain of the semi-invariant TCR strongly influences TCR avidity for CD1d: glycolipid complexes. This result is currently being exploited to investigate the role of TCR avidity in positive and negative selection of NKT cells during thymus development.

In contrast to conventional T cells NKT cells express markers normally associated with NK cells including members of the Ly-49 receptor family. However whereas NK cells express both activating and inhibitory Ly-49 receptors, NKT cells only express inhibitory family members. The group has constructed transgenic mice expressing either activating or inhibitory Ly-49 receptors in NKT cells to investigate the role of Ly-49 receptor signaling in NKT cell development. It was found that forced ligation of activating Ly-49 receptors blocks NKT cell development at an early stage, while co-expression of an inhibitory Ly-49 receptor with the same ligand specificity rescues NKT cell development. Since Ly-49 receptors utilize similar positive (ITAM) and negative (ITIM) signaling motifs as TCR, these data indicate that appropriate TCR signaling thresholds are essential for NKT cell development.

INNATE IMMUNITY GROUP

The Innate Immunity Group led by Dr. Werner Held studies the development and function of natural killer (NK) cells. NK cells play important roles for innate immunity to infection and tumor cells. During the last year, the group reported that development of NK cells is dependent on the transcription factor TCF-1 (T cell factor 1). One of the targets of TCF-1 is Ly-49A, an inhibitory NK cell receptor specific for Major Histocompatibility Complex (MHC) class I molecules. This receptor normally prevents NK cell auto-aggression and enables NK cell reactions to aberrant cells lacking MHC class I molecules. Inhibitory NK cell receptors are also expressed on T cells, in particular on CD8+ memory T cells. In contrast to the constitutive expression on NK cells, the group found that Ly-49A expression was induced after the stimulation of auto-reactive TCR $\alpha\beta$ T cells with their cognate self-antigen. Ly-49A expression substantially reduced the activation of auto-reactive T cells. Thus induction of inhibitory NK cell receptors on T cells contributes to self-tolerance.

MOLECULAR IMMUNOLOGY GROUP

The Molecular Immunology Group led by Dr. Immanuel Luescher continues to investigate the molecular mechanisms involved in CD8 cytolytic T lymphocyte (CTL) recognition of, and activation by, peptide-MHC (pMHC) complexes. The current research effort deals with the preparation of well-defined soluble murine and human MHC class I (H2-Kd and HLA-A2) and their testing on cloned CTL of appropriate specificity. Using soluble Kd-PbCS(ABA) complexes, the group tested a family of dimers containing spacers of different length. Interestingly, dimers containing short linkers (3-30 Å) efficiently bound to and activated cloned CTL, whereas those containing long spacers (> 80 Å) did not. Based on fluorescence resonance energy transfer (FRET) experiments and computer assisted docking experiments, the group has described a high avidity binary antigen recognition mode, in which two pMHC complexes engage two TCR and CD8 molecules in an anti-parallel fashion. In addition, short dimers (as well as tetramers and octamers) induced rapid and vigorous apoptosis of antigen-specific CTL. This apoptosis was not perforin/granzyme, Fas or caspase dependent, but seemed to be mediated by pro-apoptotic molecules of the Bcl2 family, most likely BNIP3. Finally, long dimers (and tetramers) not only failed to activate antigen-specific CTL, but effectively inhibited their activation by agonists. The mechanism for this new type of antagonism seems to be interference with TCR dimerization/aggregation.

VIRAL IMMUNOLOGY GROUP

The Viral Immunology Group led by Dr. Hans Acha-Orbea has continued the study of the development and function of germinal centers in response to chronic viral infections. An important role of the lymph node draining the site of mouse mammary tumor virus (MMTV) injection in the maintenance of a chronic neutralizing antibody response was found. Surgically removing the draining lymph node after establishment of the germinal center reaction led to complete loss of neutralizing antibodies despite comparable systemic spread of MMTV infected lymphocytes. Importantly, in the absence of neutralization, only the exocrine organs (mammary gland, salivary gland and pancreas) and skin showed strikingly increased infection resulting in accelerated mammary tumor development. Therefore, the antigen deposits in the draining lymph node and the chronic immune response in this site appear to be crucial for the maintenance of a strong neutralizing immune response. The group is now investigating the co-stimulation requirements for the maintenance of chronic germinal center reactions. For this purpose the group produced antibodies and recombinant molecules as well as generated several recombinant viruses that encode either stimulating or inhibiting co-stimulatory molecules. In parallel, genes specifically expressed in germinal centers are being cloned. For this purpose the group has isolated different germinal center subsets such as centrocytes, centroblasts or follicular dendritic cells and analyzed their gene expression program.

ANTIGEN PROCESSING GROUP

The Antigen Processing Group led by Dr. Frédéric Lévy is involved in the analysis of the intracellular events that contribute to the production of CTL-defined tumor antigens. The group has shown previously that the HLA-A*0201-restricted immunodominant peptide derived from Melan-A is inefficiently processed in cells expressing the immunoproteasome, a type of proteasome that is constitutively expressed in dendritic cells and induced in many other cells upon exposure to IFN- γ . Immunization of HLA-A2 transgenic mice with recombinant vectors expressing the minimal antigenic sequence of Melan-A induced a potent CTL response. In contrast, a modest response was detected after immunization with vectors expressing the full length Melan-A protein. We are currently evaluating whether the immunoproteasome contributes to this phenomenon.

Although the proteasomes produce the C-termini of most antigenic peptides, they do not always generate the appropriate N-termini. Those intermediates carry N-terminal extensions of varying length. However, for the HLA-A*0201-restricted Melan-A peptide, the proteasomes generate both N-terminally extended intermediates and fully processed peptides. In such cases, the group found that the fully processed proteasomal product was preferentially selected for presentation by HLA-A*0201 molecules. This result implies that immunization with vectors coding for minimal Melan-A peptides should be favored.

MOLECULAR TUMOR IMMUNOLOGY GROUP

The current research efforts of the Molecular Tumor Immunology group are devoted to the characterization of human tumor antigens that are recognized by CD8⁺ T cells from melanoma patients. An ongoing project is the delineation of the function of the melanocyte lineage specific protein Melan-A, a small transmembrane protein that is the target of vaccine trials in the Lausanne Branch. Previous studies by the group have revealed that its subcellular localization is distinct from typical melanosomal proteins, such as tyrosinase, as Melan-A accumulates in the trans-Golgi network and in early stage melanosomes. Furthermore, the group found that Melan-A is palmitoylated, suggesting the association with particular membrane subdomains. To understand the role of Melan-A in melanocytic cells, *in vitro* knock-down cells have been generated using lentivirus-delivered siRNA. The phenotype of the cells thus generated points to a possible negative role of Melan-A in pigmentation. In addition, the group has found that Melan-A is mono-ubiquitylated, a modification that can act as a sorting signal for the endocytic pathway. The implications of this modification on the protein function, localization and stability are currently under investigation.

A major breakthrough in melanoma research has been the recent discovery of a somatic point mutation in the BRAF gene in over 65% of tumors. BRAF is a serine/threonine kinase in the MAPK pathway mediating cellular responses to growth signals. The mutated BRAF protein possessed constitutively elevated kinase activity. The presence of the mutation may also have immunological consequences, as it may lead to the generation of novel or more antigenic CTL epitopes. As a background study to explore this hypothesis, a series of melanomas has been screened for the common BRAF mutation. The group could confirm that the majority of cutaneous melanoma metastases harbour the mutation. Surprisingly, all of the primary and metastatic uveal melanoma tumors tested carried a wild-type sequence, indicating a different etiology for cutaneous and uveal melanocytic tumors. Despite the absence of BRAF mutations, uveal melanomas, similar to their cutaneous counterparts, displayed an activated MAPK pathway, suggesting that deregulation of this pathway may be a hallmark of melanocytic transformation.

A distinct project, carried out in collaboration with the LICR Information Technology Office led by Dr. Victor Jongeneel and the Swiss Institute of Bioinformatics, has used and developed molecular modeling techniques, such as homology modeling, molecular dynamics and free energy simulations, to design optimized peptides for cancer vaccines and construct models of T cell receptor-peptide-MHC complexes. This approach has been applied to the well defined Melan-A peptide presented by HLA-A2. Modified peptide candidates have been identified that exhibit increased HLA-A2 binding and protease resistance without alteration of the conformation adopted by the native peptide in the HLA-A2 groove. The best candidates have been synthesized and are being tested for efficient recognition by *bona fide* Melan-A specific CTL clones derived from melanoma patients.

CLINICAL TUMOR IMMUNOLOGY GROUP

The Clinical Tumor Immunology Group led by Dr. Pedro Romero is focused on the design of molecularly defined therapeutic cancer vaccines. The melanocyte/melanoma associated antigen Melan-A/MART-1 is a well defined model system for these studies. An immunodominant peptide binding to the HLA-A2 molecule is recognized by tumor reactive T cells present at high numbers in the metastatic lesions of the majority of melanoma patients. An additional unique feature of this antigen is the presence of a high frequency of functionally naïve antigen specific CD8⁺ T cells in the peripheral blood of the majority HLA-A2 individuals, independent of the presence of a malignant melanoma lesion. This provides a convenient baseline to monitor the frequency as well as the functional differentiation of Melan-A-specific T cells. As part of the Institute Clinical Trials program, a series of closely related phase I clinical trials with Melan-A peptide based vaccines have been carried out in collaboration with the Multidisciplinary Oncology Center (CePO, CHUV, Lausanne), the Division of Oncology of the University Hospital in Geneva (HUG) and the Brussels Branch. Forty nine patients with Melan-A⁺ high risk stage III and IV melanoma were vaccinated with Melan-A peptide +/- adjuvant +/- low dose recombinant IL-2. The peptide-specific T cell response in blood was measured directly *ex vivo* by both multiparameter flow cytometry combining HLA-A2/Melan-A peptide multimers and cell surface markers and direct IFN- γ ELISPOT. These studies indicated that repeated vaccination with Melan-A peptide and an incomplete Freund's adjuvant equivalent approved for human use frequently leads to sustained *ex vivo* detectable CD8 T cell responses. Two new, but related, phase I clinical trials were initiated this year. The first is designed to test the immunogenicity of the same peptide vaccine + Montanide combined with synthetic oligodeoxynucleotides containing bacterial DNA CpG motifs, a new adjuvant that activates plasmacytoid dendritic cells, NK and B cells

via Toll-like receptor-9. Preliminary results indicate that addition of the CpG-ODN to the vaccine greatly enhances its ability to induce rapid A2/Melan-A peptide multimer+ CD8+ T cell responses. The second trial will determine the immunogenicity of the same Melan-A peptide admixed with the outer surface membrane protein of Gram negative bacteria OMPA-40, a new adjuvant that activates dendritic cells via the Toll-like receptor-2. The goal of these trials is to make stepwise progress towards the optimization of synthetic cancer vaccines.

The group is also involved in the analysis of the naturally acquired CTL responses directed against well defined tumor antigens. In addition to the response to Melan-A, the responses to other melanoma associated tumor antigens such as tyrosinase, gp100, NY-ESO-1/LAGE-1, MAGE-A10 and SSX-2 are investigated. The aim of these studies is to obtain a complete quantitative and functional assessment of antigen-specific T cells freshly isolated from human tissues. In this regard, the group demonstrated that HLA-A2 multimers bearing two mutations in the $\alpha 3$ domain, which abolish their interaction with the CD8 coreceptor on T cells, selectively bind to high avidity T cells. Thus, these reagents may be well suited to directly visualize T cells expressing high avidity T cell receptors, a critical parameter in vaccination. The group has also initiated the analysis of human CD4 T cell responses to well defined tumor and virus-derived antigens. To increase the analytical power, new PCR-based techniques are now routinely applied to the study of single antigen-specific T cells. These include T cell receptor CDR3-spectratyping and sequencing and single cell PCR. The latter is used to assess the expression of transcripts encoding molecules associated with T cell functions. In a separate project, collaborative work with the groups of Dr. H. R. MacDonald and Dr. W. Held investigates the role of human NKT cell responses to CD1/ α -galactosylceramide in tumor immunity. Altogether, the results will be of great help for the accurate monitoring and development of new cancer vaccines.

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