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TUMOR IMMUNOLOGY AND ANTIGEN PROCESSING

The group follows three main lines of research. The first focuses on the processing of tumor antigens, studying the role of the proteasome and other proteases in the production of tumor antigenic peptides. The second studies mechanisms whereby tumors resist immune rejection. The third develops new preclinical models for cancer immunotherapy. The long term goal of these projects is to better understand the interaction of tumors with the immune system and devise strategies to improve the efficacy of cancer vaccines.

PEPTIDE SPLICING BY THE PROTEASOME

A. Dalet, V. Stroobant, N. Vigneron

Tumor antigens relevant for cancer immunotherapy consist of peptides presented by MHC class I molecules and derived from intracellular tumor proteins. They result from the degradation of these proteins, which is mainly exerted by the proteasome. We have described a new mode of production of an-

tigenic peptides by the proteasome, which involves the splicing of peptide fragments, either in the normal or the reverse order (1, 2). In the two cases we initially described, we showed that splicing occurs in the proteasome catalytic chamber through a reaction of transpeptidation involving an acyl-enzyme intermediate (Figure 1). We have now demonstrated that the same mechanism accounts for the splicing of a third spliced peptide, derived from FGF5, despite the fact that the fragments to splice are distant from each other by 40 amino acids (3). We also compared the efficiency of splicing by

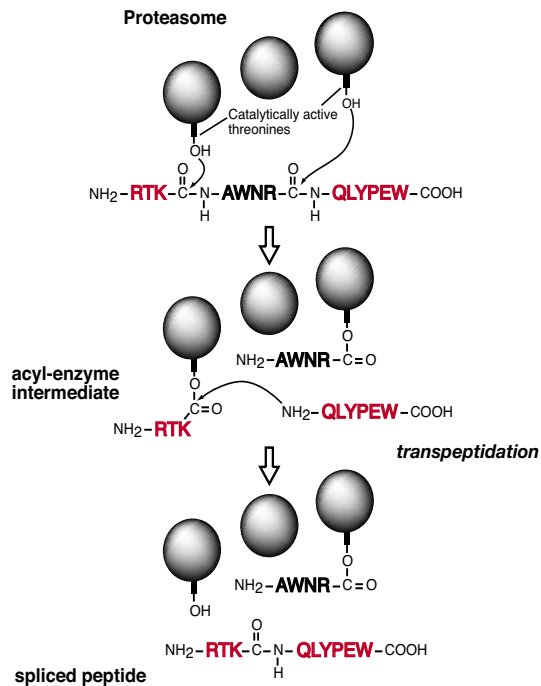


Figure 1. Model of the peptide-splicing reaction in the proteasome. The active site of the catalytic subunits of the proteasome is made up of the side-chain of a threonine residue, which initiates proteolysis by performing a nucleophilic attack on the carbonyl group of the peptide bond. An acyl-enzyme intermediate is formed, which is then liberated by hydrolysis. In the peptide-splicing reaction, a second peptide fragment appears to compete with water molecules for performing a nucleophilic attack on the acyl-enzyme intermediate, resulting in a transpeptidation reaction producing the spliced peptide. Experimental support for this model of reverse proteolysis includes evidence that the energy required to create the new peptide bond is recovered from the peptide bond that is cleaved at the amino-terminus of the excised fragment, and that the amino-terminus of the other fragment needs to be free for transpeptidation to occur.

the standard proteasome and the immunoproteasome, which is found in antigen-presenting cells and cells exposed to interferon-gamma, and contains three inducible catalytic subunits $\beta 1i$, $\beta 2i$ and $\beta 5i$ instead of the standard catalytic subunits $\beta 1$, $\beta 2$ and $\beta 5$. We found that both proteasomes were able to splice peptides, but their relative efficiency was different for each peptide, depending on the major cleavage sites. This is consistent with the transpeptidation model of splicing.

NEW PROTEASOME TYPES THAT ARE INTERMEDIATE BETWEEN THE STANDARD PROTEASOME AND THE IMMUNOPROTEASOME

B. Guillaume, V. Stroobant, A. Busse

Using a series of novel antibodies recognizing catalytic subunits of the human proteasome in their native conformation, we also identified proteasomes that are intermediate between the standard proteasome and the immunoproteasome. They contain only one ($\beta 5i$) or two ($\beta 1i$ and $\beta 5i$) of the three inducible catalytic subunits of the immunoproteasome. These intermediate proteasomes represent 30-54% of the proteasome content of human liver, colon, small intestine and kidney. They are also present in human tumor cells and dendritic cells. We studied the processing of a series of antigenic peptides by these intermediate proteasomes, and identified two tumor antigens that are processed exclusively either by intermediate proteasomes $\beta 5i$ or by intermediate proteasomes $\beta 1i$ - $\beta 5i$.

PRODUCTION OF AN ANTIGENIC PEPTIDE BY INSULIN-DEGRADING ENZYME

N. Parmentier, V. Stroobant

We studied a proteasome-independent peptide derived from tumor protein MAGE-A3, and we identified insulin-degrading enzyme as the protease producing both the C-terminus and the N-terminus of this peptide (4). This peptide, with sequence EVDPIGHLY, is presented by HLA-A1 and has been widely used in clinical trials of cancer vaccines. Insulin-degrading enzyme is a cytosolic metallopeptidase not previously known to play a role in the class I processing pathway. Cytotoxic T lymphocyte recognition of tumor cells was reduced after metallopeptidase inhibition or IDE silencing.

Separate inhibition of the metallopeptidase and the proteasome impaired degradation of MAGE-A3 proteins, and simultaneous inhibition of both further stabilized MAGE-A3 proteins. These results suggest that MAGE-A3 proteins are degraded along two parallel pathways that involve either the proteasome or IDE and produce different sets of antigenic peptides presented by MHC class I molecules.

MODULATION OF TUMOR ANTIGEN EXPRESSION BY INFLAMMATORY CYTOKINES

E. De Plaen, O. Kholmanskikh

We recently observed that treating some melanoma cell lines with the inflammatory cytokine IL-1 β leads to a 4- to 10-fold decrease in the level of Microphthalmia-associated transcription factor (MITF-M) (5). This effect is NF- κ B and JNK-dependent. MITF-M regulates the expression of melanocyte differentiation genes such as Melan-A, tyrosinase and gp100, which encode antigens recognized on melanoma cells by autologous cytolytic T lymphocytes (CTL). Accordingly, treating some melanoma cells with IL-1 β reduced by 40-100% their ability to activate such anti-melanoma CTL.

TUMORAL IMMUNE RESISTANCE THROUGH TRYPTOPHAN DEGRADATION

L. Pilotte, P. Larrieu, V. Stroobant

An important factor limiting the efficacy of immunotherapy is the development of mechanisms allowing tumors to resist or escape immune rejection. Immune resistance mechanisms often involve modulation of the tumoral microenvironment resulting in local immunosuppression. We described one such mechanism, based on the expression by tumor

cells of Indoleamine 2,3-dioxygenase (IDO), a tryptophan-degrading enzyme inducing a local tryptophan depletion that severely affects T lymphocyte proliferation (6). Our data in a pre-clinical model indicate that the efficacy of therapeutic vaccination of cancer patients could be improved by concomitant administration of an IDO inhibitor. In collaboration with the group of Olivier Michielin in Lausanne, we described new compounds able to inhibit IDO in the micromolar range, not only in enzymatic assays but also in cellular assays (7). These compounds will be further optimized with the goal of developing drug candidates. In parallel, a large effort was launched in collaboration with academic and industrial partners to identify IDO inhibitors by high-throughput screening of a chemical library and by structure-based drug design.

We have produced a monoclonal antibody against human IDO, which we used to characterize IDO expression in normal and tumoral tissues. Although others reported high expression of IDO in dendritic cells of murine tumor-draining lymph nodes, our results in humans indicate that a subset of mature human dendritic cells express IDO but these cells are present in normal lymph nodes and not enriched in tumor-draining lymph nodes. However, we observed expression of IDO in a high proportion of human tumors, confirming our initial observation.

NEW PRECLINICAL MODELS FOR CANCER IMMUNOTHERAPY

C. Powis de Tenbosche, (in collaboration with C. Uyttenbove, de Duve Institute and A.-M. Schmitt-Verbulst, CIML, Marseille)

We have devised a mouse melanoma model, in which we can induce melanoma in 70% of mice injected with tamoxifen (8). These tumors express the tumor antigen encoded by cancer-germline gene P1A. They can be either highly

pigmented and indolent, or unpigmented and highly aggressive. We observed a correlation between aggressive tumor progression and the occurrence of exacerbated systemic inflammation, involving disruption of secondary lymphoid organs, extramedullary hematopoiesis and accumulation of immature myeloid cells, which may contribute to tumoral immune resistance (9).

Cancer-germline genes, which encode tumor antigens of the MAGE-type, are expressed at a low level in the thymus, possibly inducing some level of central immune tolerance that may explain the poor immunogenicity of many of the antigens encoded by these genes. To address this issue, we produced mice that are knockout for cancer-germline gene P1A. These mice are normal and fertile. Their ability to develop an immune response against the P1A-encoded antigen is slightly higher than the wild-type mice, resulting in a better ability to reject P1A-expressing tumors spontaneously. Analysis of the repertoire of TCR genes revealed some differences in V β gene usage. This result is consistent with the deletion of high affinity T cells recognizing P1A-encoded antigens in wild-type mice. We conclude that there is a limited central tolerance towards antigens encoded by cancer-germline genes.

TRANSCRIPTOMIC STUDIES IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) AND RHEUMATOID ARTHRITIS (RA)

B. Lauvery, I. Gutierrez-Roelens, V. Badot, A.-L. Maudoux (in collaboration with F. Housiau, Unité de Rhumatologie)

SLE is a systemic autoimmune disorder of unknown etiology, characterized by the activation of autoreactive CD4 T and B cells directed against constituents of the chromatin and the production of pathogenic antinuclear antibodies. Recently, several groups identified

a characteristic interferon signature in PBMC from SLE patients, i.e. the over-expression of genes induced by type I interferons. We compared SLE synovitis and the synovitis of osteoarthritis (OA) and RA using high-density oligonucleotide spotted microarrays. Our results indicate that SLE arthritis is characterized by a very specific molecular signature that is distinct from that of OA and RA, with up-regulation of interferon (IFN)-inducible genes and down-regulation of genes involved in extracellular matrix (ECM) homeostasis. The latter observation is probably associated with the less destructive character of SLE compared to RA and OA. These results have immediate clinical applications for the differential diagnosis of arthritis.

We also performed global gene expression studies on synovial biopsies from RA patients treated with TNF blockers. We identified gene signatures in pre-treatment synovial tissue that predict the absence of response to TNF blockade. Not surprisingly, these genes can be induced in synovial cells by other inflammatory cytokines (such as IL-1b or IL-17), alone or in combination with TNF- α (10). These observations can be useful to guide therapeutic decisions.

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