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[11.00–11.30]

Tumor rejection in anti-idiotypic DNA vaccination is antibody-mediated

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The particular combination of the two variable (V) regions of Ig define the idiotype (Id), a unique antigenic structural feature that constitute a clonal signature of individual membrane-Ig$^+$ B cells. DNA vaccination with the idiotype (Id) derived from tumour B-cells immunoglobulins is a validated strategy to induce anti-Id antibodies and tumour protection in several mouse lymphoma models. The role of anti-Id antibodies on the mechanism of rejection has not been fully understood. We have investigated the structural basis of the idiotypic/anti-idiotypic antibodies interaction following DNA immunisation with scFvs expressing either the complete lymphoma Id, or Ids containing only one of the two lymphoma V regions associated to an irrelevant partner. Constructs encoding a secretory form of the scFv were used as immunogens to induce anti-Id antibodies. In order to obtain proper display of the immunising Id, the same scFvs were expressed as membrane-bound molecules on the surface of mammalian cells. Analysis of immune sera on the membrane-displayed idiotypes revealed that DNA immunisation induced a polyclonal antibody response restricted to conformational combined epitopes formed by the parental V_L/V_H association. No detectable reactivity towards chimeric scFvs containing only one of the two immunising V regions were detected, indicating that the response against combined V_L/V_H determinants is highly dominant. Remarkably, the same immunogen delivered as scFv protein induced antibodies directed also against chain specific determinants. Thus, presentation of properly folded Ids results in a highly specific antibody response directed exclusively to private idiotypic determinants of the immunising V_L/V_H combination. We took advantage of these findings to demonstrate that the mechanism of tumour rejection in the anti-idiotypic response induced by genetic immunisation is entirely dependent on the presence of antibodies. Indeed, only animals that elicited a specific anti-Id response (vaccinated with the lymphoma Id) were protected against tumour challenge, whereas animals vaccinated with one or both chimeric constructs died despite the exposure to all putative tumour-Id CD8$^+$ T cell epitopes.

[11.30–12.00]

Engineering low affinity IgM antibodies into high affinity, fully human IgG molecules

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Angiogenesis is often accompanied by extensive remodeling of the basement membrane that lines existing blood vessels. Although the precise role of this degradation process is not known, it is thought to facilitate the migration of endothelial cells and the release of growth factors to the site of blood vessel formation. One of the major components of the basement membrane is collagen type IV whose proteolytic cleavage during angiogenesis reveals a cryptic site not exposed in the native molecule. This epitope appears to be specifically exposed in the basement membrane of angiogenic blood vessels but is not detected in association with quiescent vessels. Mouse monoclonal antibodies against cryptic epitopes of collagen molecules were generated by substractive immunization. One antibody, HUIV26, specifically recognizes proteolyzed or denatured collagen IV but not native collagen and is a

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potent inhibitor of angiogenesis and tumor growth in animal models. Unfortunately, antibodies with the desired epitope recognition were all IgM molecules displaying inherently low affinity. HUIV26, along with a second antibody recognizing a cryptic epitope found on both collagen I and IV, were subjected to directed evolution *in vitro* in order to improve their clinical profile. The CDRs of both mouse antibodies were engineered to substantially improve affinity in the context of a fully human framework. The intrinsic low affinities of the original mouse IgM antibodies were increased by three orders of magnitude while preserving their respective epitope recognition. The generation of high affinity variants enabled the conversion of pentameric IgM antibodies into IgG molecules more suitable for clinical development.

[12.00–12.30]

Human membrane immunoglobulins are stabilized by inter-chain disulfide bonds within the extracellular membrane-proximal domain

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Immunoglobulins can be expressed as secretory or membrane-bound proteins, depending on the stage of differentiation of B-cells. Membrane immunoglobulins differ from the secretory form for the presence of three additional C-terminal domains in the heavy chain, namely the extracellular membrane-proximal domain (EMPD), the transmembrane domain (TMD), and the cytoplasmic domain.

An interesting structural feature of human γ, α and ε EMPDs is the presence of cysteine residues, albeit in different numbers. Immunoglobulins heavy chains covalently associate through inter-chain disulfide bonds located in the Fc fragment. Using a genetic strategy, we investigated whether EMPD cysteines are also involved in the formation of inter-chain disulfide bridges.

Shortened versions of human membrane immunoglobulins, depleted of cysteines known to form intermolecular disulfide bonds, were constructed, and expressed on the surface of a B-cell line. These recombinant membrane proteins contain a single-chain fragment of variable regions (scFv) linked to a heavy chain dimerizing domain (CH3 for α and γ or CH4 for ε isotypes), followed by the corresponding EMP, TM, and cytoplasmic domains. We analyzed the EMPDs of the isotype γ, α, and the two ε isoforms (characterized by a short and a long EMPD), that contain cystein residues.

We found that the single cysteine within α, γ, and the short version of ε EMPD form an inter-chain disulfide bond. Of the four cysteines contained in the ε-long EMPD, two are involved in inter-chain bridges, while the remaining two are forming an intra-chain bond. Inter-chain bonds were also demonstrated within EMPDs of complete membrane immunoglobulins.

Work in progress has revealed that, in addition to this important structural function, the EMPD is emerging as a domain with a relevant role in the assembly and signaling capacity of the B-cell receptor.

[12.30–13.00]

Generating human monoclonal antibodies from human cells engrafted into SCID mice

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Xenerex is generating fully human antibodies from human cells engrafted in severe combined immunodeficient (SCID) mice (DE Mosier et al., *Nature* 1998 335:256). Immunocompetent human cells obtained from peripheral blood, spleen or tonsils are routinely introduced into SCID mice. Human lymphocytes from individuals who have been previously exposed to an antigen can be used to generate antibodies with essentially identical properties as those found in the serum of the lymphocyte donor. We have refined the method for the exposure of the human cells to antigen, both, *in vitro* and *in vivo*, which results in the generation of specific human antibodies. Data will be presented to demonstrate that antibodies directed towards soluble human proteins, membrane bound human proteins and infectious disease targets can be generated. ELIASPOT assays are used to determine the frequency of human antigen specific cells recovered from selected mice. Furthermore, we will present our approach for the isolation of antigen specific human B cells and the rescuing of antibodies that have desired characteristics by recombinant cloning and expression.