Cellular signalling in T lymphocytes

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T-cell activation requires two signals: an antigen-specific signal and an additional amplifying signal. The former is delivered via the T-cell antigen receptor (TcR), which comprises a disulphide-linked heterodimer associated non-covalently with the CD3 complex. The CD3 component consists of at least four distinct polypeptides called γ, δ, ϵ and ζ. Several receptors are capable of delivering amplifying signals. The CD2 antigen represents a prime example of such a receptor. It is a single, glycosylated polypeptide that binds to the LFA3 glycoprotein. Other T-cell surface glycoproteins implicated in T cell activation include the CD4, CD8 and CD45 antigens.

Signals transduced via CD2 and the TcR to the nucleus result in activation of gene expression and eventually in cell division. A number of intermediate events have been defined at the level of the inner surface of the T-cell plasma membrane. These include the participation of G proteins and particularly of phosphorylation mediated by protein kinase C and tyrosine kinases. Tyrosine phosphorylation is thought to be especially important and may in part be regulated by the CD45 antigen. The details of the pathway(s) between the plasma membrane and nucleus are mysterious but probably involve activation of transcription factors that activate T-cell genes. The enhancers and promoters of several T-cell genes have been characterised and provide a basis for understanding T-cell activation at the transcriptional level.

Tumour-associated and tumour-specific antigens as targets of immune intervention

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There is growing evidence that tumour cells may express viral proteins, oncogenes or somatic mutations that generate new peptide epitopes that can be recognized by class I restricted cytotoxic T-cells. A T-cell response to the tumour-specific antigens may be detected in tumour infiltrating lymphocytes, but is usually insufficient to cause tumour regression. The failure of the immune response is probably related to the inefficiency of tumour cells as antigen presenting cells. Thus tumour cells may fail to trigger an effective cytotoxic response because: (i) they lack a costimulatory activity required for T-cell priming and (ii) they lack helper determinants required to trigger helper T-cells, which in turn are necessary to boost the cytotoxic T-cell response.

In principle this problem could be solved by targetting to the tumour cells activated T-cells. In collaboration with M.I. Colnaghi (Istituto Tumori, Milan) we have produced a hybrid anti-CD3/anti-ovarian carcinoma antibody by fusing an anti-CD3 hybridoma with a hybridoma specific for an ovarian carcinoma-associated antigen (MOV18). This hybrid antibody can be used to arm *in vitro* polyclonally activated T-cells that acquire and retain for up to two days the capacity to kill ovarian carcinoma cells. The infusion of activated T-cells armed with this bifunctional antibody may be effective not only in inducing tumour cell killing, but also may act as a powerful boost for tumour-specific antigens. A clinical trial, which is underway, will establish whether it is possible to focus T helper cells to a tumour-associated antigen to boost the cytotoxic response to a tumour-specific antigen.

Session 2. Chairman: Sir Walter Bodmer, London, UK

Building antibodies from their genes

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Recently it has proved possible to clone the genes encoding immunoglobulin variable regions directly from mRNA or chromosomal DNA of lymphocytes using the polymerase chain reaction (R Orlandi *et al. Proc Natl Acad Sci USA* 1989, **86**, 3833–3837). The genes can be expressed from bacteria as single domains (Ward *et al. Nature* 1989, **341**, 544–546) or as F(v) or F(ab) fragments with antigen binding activities. Strategies for building such antibody fragments have been developed.

New cytotoxic agents created by the fusion of cell targetting and Pseudomonas toxin genes

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Immunotoxins have been made conventionally by attaching a toxin to a monoclonal antibody using chemical cross-linking reagents. We have developed a new approach to toxin based cDNAs encoding growth factors, other cell recognition proteins, or recombinant single chain antibodies recombinantly attached to mutant non-toxic forms of Pseudomonas exotoxin. Native Pseudomonas toxin is a 66 000 molecular weight protein that is made up of three domains. Domain I is the cell binding protein, domain II promotes translocation into the cytosol and domain III contains the ADP-ribosylating function responsible for cell death. To create cell-specific chimeric toxins, we have replaced domain I with TGF, IL2, IL4, IL6, DC4 or a single chain antibody against the interleukin 2 receptor and created cytotoxic molecules that kill cells with the appropriate receptors on their surfaces. These chimeric toxins are active on tissue culture cell lines and in animal models.

Novel antibodies: their prediction and design

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Although a great deal is known about the genetic basis of antibody diversity, our understanding of the structural origins of antibody–antigen interactions remains limited. The prediction that the combining site of an antibody molecule is created by the folding of three hypervariable, or complementarity determining regions (CDR) in each heavy and light chain to form a three-dimensional binding site, has been confirmed by X-ray crystallographic analysis of both F(ab)-hapten and F(ab) protein antigen complexes.

A knowledge of the rules involved in defining the three dimensional structure of these CDRs and in specifying those residues making specific interactions with a particular antigen, would allow the design and manipulation of the CDR sequences by protein engineering techniques. Progress in the construction of algorithms that combine "knowledge based" prediction and *ab initio* calculations for the modelling of the antibody combining site will be presented. In addition, practical uses of such methods in the design of novel antibody features, such as the ability to bind metal ions or catalyse chemical reactions, will be described with particular reference to medical applications.