The EMBO Medal for 1988 has been awarded to
Dr Antonio Lanzavecchia of the Basel Institute for
Immunology, Switzerland. Dr Lanzavecchia receives the
Medal for his work on antigen presentation by B cells
and the quantitation of human B-cell responses by
antigen-specific alloreactive T cells.

EMBO MEDAL REVIEW
Clonal sketches of the immune response

A. Lanzavecchia

Basel Institute for Immunology, Grenzacherstrasse 487, Postfach, CH-4005 Basel, Switzerland

Cell clones: revealing tools for immunology

While trying to concentrate in some pages a few years of my scientific life, I realized once more how important it is to make the right decision at the right moment. In 1980 I had just moved to a new institute in Genova and started working in a new field. I had left behind my research activities in clinical immunology and for several months I agonized over what to do next, helped and influenced by my new colleagues. Franco Celada, my friend and the director of the institute, set only two conditions: my territory should be in the centre of immunology and it should deal with real immune responses, i.e. antigen driven. In a short time my future research activity was decided—I was interested in the cellular interactions involved in the immune response.

I wish I could say that I started to work using the human model because of my medical background. In fact there was really no choice, since in the institute where I was, the most abundant kind of experimental animals available were human beings. Human beings are obviously not the right experimental animals for in vivo manipulations, so I started to do my work in vitro using peripheral blood cells obtained from my colleagues.

Indeed, in the seventies cellular immunology had moved to a great extent towards in vitro studies with the aim of resolving the different components of the immune response. This approach suffered from one major limitation. Since the frequency of antigen specific lymphocytes is very low, it was particularly difficult to make them interact in the artificial environment of a tissue culture plate. Therefore all attempts to recreate in vitro the specific response to antigen using isolated ex vivo cells soon became a kind of academic exercise. To bypass the problem of the low frequency of antigen-specific cells, some investigators used mitogens which can activate a large fraction of lymphocytes irrespective of their specificities. Although this turned out to be a very fruitful way to study T and B cell activation, it could not help to elucidate the very fundamental question of how lymphocytes recognize foreign antigens.

Rather than trying to recreate the immune response in vitro, the time was right for a further radicalization of the in vitro approach to cellular immunology. By 1981 I was convinced that the promising approach was to study homogeneous clonal populations of cell stimulated by antigen and I started to work out a way of cloning T cells.

Here I must acknowledge my luck for having chosen the human system, because isolating and growing human T and B cell clones turned out to be (at least in my hands) rather easy. Although at the beginning I was very sceptical, because

of difficulties reported in other labs, I soon realized that most T cells can be cloned and expanded in vitro to a practically unlimited number of cells that retain the original specificity and effector function. All that is necessary is to provide them with their growth factor Interleukin 2 (IL-2) and periodically renew their sensitivity to IL-2 by means of the mitogen phytohaemagglutinin in the presence of irradiated accessory cells.

The use of T cell clones in vitro has helped to unravel many aspects of the immune response, such as the nature of the antigen recognized by T cells and the role of specific receptors in cellular interactions. In all cases the results are unambiguous and rarely influenced by artifacts introduced by tissue culture.

In the following pages I will describe some experiments which address some questions in immunology. These are sketches in the sense that they provide not a complete picture, but hopefully a clear and authentic glimpse of how a complex biological system works.

The role of membrane Ig in antigen presentation by B cells

It is well established that specific T and B cells must interact with each other in the presence of antigen in order to obtain an antibody response. In an influential series of experiments, Mitchison demonstrated that B and T cells interact by recognizing different determinants on the antigen (called hapten and carrier) and that, for B–T interaction to occur, hapten and carrier must be physically linked. To account for these findings he proposed that B and T cells interact via an 'antigen bridge' with antigen engaging both their specific receptors simultaneously (Mitchison, 1971).

This proposal soon met with several difficulties since it became increasingly clear that T cells recognize only degraded fragments of antigen in association with MHC class II molecules (Rosenthal and Schevach, 1973). Indeed most native antigens must be captured and degraded (processed) by macrophages (called antigen presenting cells, APCs) and the fragments of antigen must be associated with class II molecules in order to be recognized by T cells (Unanue et al., 1984; Babbitt et al., 1985). In addition, it became clear that the interaction between T cells and B cells (as the interaction between T cells and APCs) is class II restricted (Katz et al., 1973). This presents us with a paradox because B cells bind antigens in their native conformation using membrane immunoglobulins (mIg). How could T cells that recognize processed antigen associated with class II see with the same receptor native antigen bound to mIg on B cells plus class II? This paradox could be resolved by assuming (Kakiuchi et al., 1983) that specific B cells would use their mIg only to capture antigen, and would subsequently process and present it to T cells in an MHC-restricted fashion as do conventional APCs; in other words sequential rather than simultaneous recognition.

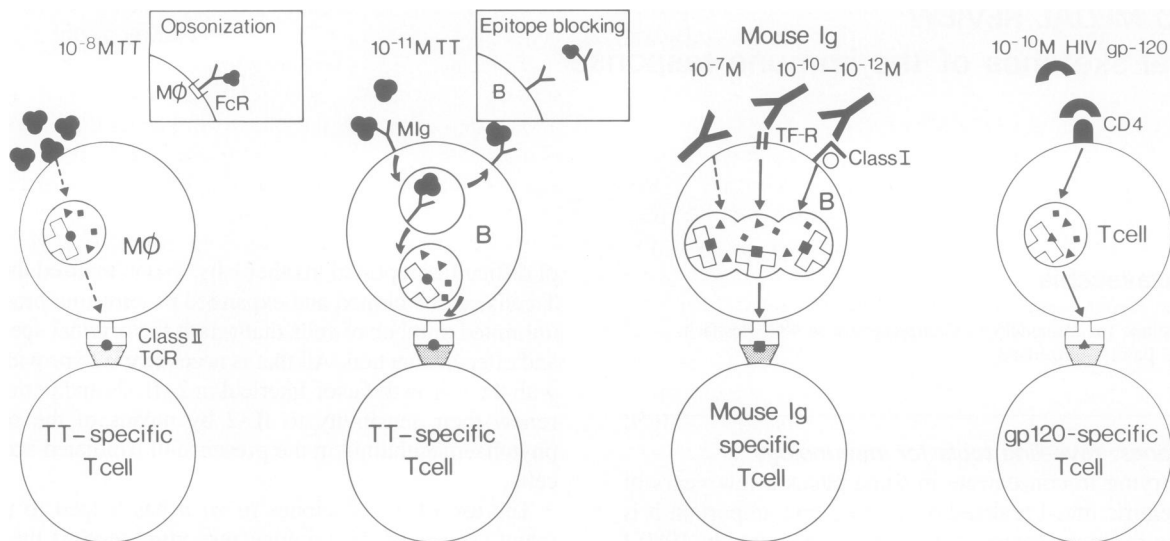The problem of studying the interaction between antigen-

Fig. 1. The capture of soluble antigens for class II-restricted presentation. The concentration of antigen required for triggering T cells is dependent on the way in which the antigen is captured. (**i**) Macrophages that pick up TT by pinocytosis require high antigen concentrations. (**ii**) TT-specific B cells use mIg to efficiently capture TT and therefore effectively present it at concentrations 10^3-fold lower than those required by macrophages. In both cases TT is processed intracellularly and presented in association with class II. Soluble antibodies opsonize antigen and facilitate its capture by Fc receptor-positive cells and, at the same time, compete with mIg on B cells for binding to a given epitope. (**iii**) Antigens can be taken up for efficient presentation through cellular receptor different from mIg. In this case mouse Ig that bind to TF-R or class I can be efficiently picked up and processed by B cells and presented to mouse Ig specific T cells. (**iv**) Human activated T cells are not effective at capturing antigens non-specifically, but can efficiently capture, process and present HIV-gp120 that binds to the CD4 molecules on their surface.

specific T and B cells is that both occur at very low frequencies *in vivo*. I felt this problem to be a challenge, and I thought that the availability of B and T cell clones from the same individual, both specific for the same nominal antigen, would make it possible to study their interaction in the presence of antigen. By chance, a colleague had just received a boost of Tetanus Toxoid (TT). I asked him for a sample of his blood and infected his B cells with Epstein—Barr Virus (EBV). In this way I isolated clones of B cells that had mIgG specific for TT. From the same sample of blood I isolated TT-specific T cell clones, so I had the system to address my questions (Lanzavecchia, 1985).

The results were quite clear cut: (i) native TT bound to specific antibodies on the surface of B cells was not recognized by T cells; (ii) B cells, like conventional APCs, had to internalize and process TT in order to present it to T cells in association with MHC class II (processing of TT takes ~ 1 h at 37°C and is sensitive to protease inhibitors and lysosomotropic agents); (iii) the binding of TT to mIg on specific B cells has a dramatic effect on the efficiency of presentation. Indeed specific B cells can present TT at concentrations $10^3 - 10^4$ times lower than those required by APCs such as macrophages which are not antigen-specific. A similar difference in the efficiency of presentation between specific and non-specific B cells was also reported in different experimental systems (Kakiuchi *et al.*, 1983; Rock *et al.*, 1984; Tony *et al.*, 1985).

All these results show that the antigen bridge model has to be redrawn to encompass the sequential recognition of antigen by B and T cells and that the difference between specific B cells and conventional APCs is in the efficacy of antigen capture through mIg receptors. The implication for the antibody response is that in the presence of high antigen concentrations, such as those required to initiate an immune response, T cells will be able to interact with all APCs, while

at low antigen concentrations, that might occur later in the immune response, only specific B cells will be able to present antigen and therefore will selectively interact with T helper cells and will be induced to make antibodies.

The selective triggering of specific B cells by low antigen concentrations (resulting in the production of specific antibody) can be also demonstrated *in vitro* using TT-specific T helper clones and normal peripheral blood B cells (Lanzavecchia *et al.*, 1983).

Soluble antibodies, which are produced following specific T—B interaction, affect antigen presentation with two distinct mechanisms. On the one hand they compete with mIg for binding of antigen and therefore exert a negative feedback on B cells with the same epitope specificity (Uhr and Möller, 1968; Lanzavecchia, 1987). On the other hand, IgG antibodies can opsonize the antigen and facilitate its capture and presentation by Fc-receptor-positive macrophages, thereby boosting the T cell response (Manca *et al.*, 1988). Thus the interaction of T cells with macrophages or specific B cells is dependent on the concentration of antigen, is modulated by soluble antibodies and may take place at different times during the immune response.

If B cells can process and present antigens directly to T cells, why do we need macrophages at all? It is possible that in a naive animal antigen specific T and B cells are too rare to find each other, and macrophages (in the presence of high doses of antigen) would be a more abundant source of APC. An alternative explanation suggested by some recent experiments involving chicken B cell chimeras (Lassila *et al.*, 1988) is that activation of virgin T cells needs accessory signals that can be delivered only by macrophages.

Using TT-specific B cell clones I could compare their ability to bind antigen with their capacity to present it to T cells. It is striking that B cells present TT at concentrations 10^3 times lower than those required to saturate 50% of mIg,

i.e. in conditions where only a few molecules are bound to mIg. This number is even more surprising if one considers that the molecules of TT bound to mIg still must be processed and their fragments associated with class II, events which are not likely to be 100% efficient.

How can so few molecules of antigen bound to mIg generate enough determinants to trigger T cells? It has recently been shown that monovalent antigen bound to mIg is rapidly removed from the cell surface by receptor-mediated endocytosis, even when very few antigen receptors are occupied (Watts and Davidson, 1988). At very low concentrations of antigen this process will eventually result in the delivery to the cell of many more molecules of antigen than can be bound to the surface receptors at any one time. If there is an imbalance between uptake and loss, antigen will accumulate in the specific B cells with time and B cells would function as a 'vacuum cleaner' that can selectively trap specific antigen. Indeed, in TT-specific B cells the antigen presenting capacity increases as a function of both antigen concentration and time of exposure at 37°C. In addition, in the same B cells antigen, as seen by the T cells, can persist for extended periods of time with a half life of ~1 day (Lanzavecchia, 1987).

What fraction of antigen is accumulating in B cells? In spite of the fact that the epitopes seen by T cells do accumulate with time, studies with radiolabelled TT did not show any dramatic accumulation of labelled material inside the B cells (Watts and Davidson, 1988). Indeed most of the radioactivity is returned to the medium as TCA-soluble material. It is therefore likely that B cells degrade antigen and selectively retain and accumulate only those fragments that became bound to cellular structures. Where are the storage compartments for processed antigen? Although class II independent ways of retaining processed antigens have been postulated (Falo et al., 1986; Lakey et al., 1987), the best candidates remain class II molecules themselves, to which immunogenic peptides have been shown to bind stably (Buus et al., 1987).

What is the intracellular route taken by antigen in specific B cells after its binding to mIg and prior to its reappearance in an immunogenic form? Watts and Davidson have shown that, in specific B cells, antigen is rapidly removed from the cell surface and that within minutes a proportion of it is recycled back to the cell surface still bound to mIg. This recycled antigen however is not yet in a form recognizable by T cells. By assaying the level of cytosolic Ca^{2+} as a rapid readout of T cell triggering, we found that processed antigen appears on the cell surface only after a lag time of ~1 h (Roosnek et al., 1988). Since this lag time is relatively insensitive to the amount of antigen initially bound to the cell, it must therefore represent the time necessary for the antigen to travel through some intracellular compartments, rather than the time for it to accumulate on the cell surface.

The behaviour of mIgG in B cells may therefore be analogous to that of the transferrin receptor (TFR). Most endocytosed TFR recycles rapidly to the cell surface from peripheral endosomes. However, it has been demonstrated that a proportion returns through a different route which appears to include the Golgi apparatus (Snider and Rogers, 1985; Cresswell, 1985). Likewise, the bulk of endocytosed antigen recycles rapidly, but clearly some fraction of antigen bound to mIg must eventually enter a protease-containing compartment for processing prior to subsequent transfer to class II molecules.

It is tempting to speculate that this transfer might happen in a region of the *trans* Golgi, where newly synthesized class II molecules (devoid of exogenous peptides) have been shown to reside for some time, before being actually transported to the membrane. At this level the intersection of the endocytic pathway with the class II biosynthetic pathway would allow highly efficient transfer of peptides to class II molecules.

In addition to its effect on antigen capture, mIg do appear to affect antigen processing. In different B cell clones that recognize different epitopes of the TT molecule, different fragmentation patterns are observed (C.Watts, personal communication). A possible consequence of this is that the set of T-cell epitopes finally displayed on the cell surface might vary from one B cell to another. As already suggested there might therefore be a 'preferential pairing' between T- and B-cell epitopes (Manca et al., 1985; Ozaki and Berzofski, 1987).

In most biological systems the affinity of receptors is matched to the physiological concentration of the ligand, i.e. the receptors work in conditions where a sizeable fraction is occupied. mIg are a remarkable exception in that they effectively capture antigen when only a minor fraction ($<1:100$) of them is occupied by antigen. The surprising sensitivity of antigen-dependent T−B interaction might be explained in at least two ways: (i) by the selective accumulation of processed antigen associated with class II in the B cell and (ii) by the fact that a small number of antigen−MHC complexes might suffice to trigger T cells.

A common pathway of antigen processing for class II restricted presentation

Having established that antigens bound to mIg are efficiently processed and presented, it was natural to ask whether this intracellular pathway of antigen processing and class II-restricted presentation is unique to mIg or not. To test other pathways of internalization, one would need an antigen that can be targeted to any chosen cell surface structure. Such an antigen could be a xenogenic antibody, which can bind to different cell surface determinants on APC because of its specificity and can be recognized, after processing, by T cells because of its intrinsic antigenicity. From patients treated with mouse monoclonal antibodies (mAbs) it was possible to isolate T cell clones that recognize processed mouse Cγ or Cκ determinants in association with human class II molecules. Using these T cell clones to assay for presented antigen, and mouse mAbs specific for different cell surface determinants as antigen, it was possible to assess different molecules on APCs for their capacity to mediate antigen capture and processing. We found that mAbs directed to the TFR, class II and class I were all successfully presented and with much higher efficiency than antibodies that did not bind (Lanzavecchia et al., 1988a). Furthermore, differences in the efficiency among binding antibodies were correlated with the level of endocytosis of their target molecule (Pernis, 1985). Therefore, not only ligands bound to IgG, but also those bound to most cell surface molecules (including IgM, class I and class II, TFR etc.) end up in the same endocytic pathway leading to antigen processing and class II-restricted presentation.

The fact that the processing pathway is shared by most cell surface molecules implies that all serum proteins that are taken up by receptor-mediated endocytosis (such as transferrin via the TFR), or are present in sufficiently high

concentrations (such as albumin), will be continuously picked up, processed and their fragments associated with class II. Thus a single class II allelic product will not be an homogeneous population of molecules, but rather a constellation of similar molecules bearing different peptides derived from a number of self proteins. Indeed only a few molecules will have a binding site free for binding antigenic peptides (Buus et al., 1987). There are two consequences that arise from this situation. First there must be competition between an incoming antigen and the large number and quantity of self proteins. This may not be such a problem because: (i) the number of epitopes required for triggering a T cell may be, as already discussed, very low and (ii) there is a threshold of antigen for the immune response to begin and this threshold is rather high. The second consequence which is more crucial is that the entire constellation of peptides is going to be recognized by T cells. Therefore, since we must be tolerant to self proteins, all these self peptide — MHC complexes must be presented in the thymus for induction of self tolerance and will consequently deplete a large number of T cells. Moreover the prediction is that alloreactivity (the recognition by T cells of an allogeneic MHC product) will not be specific for the MHC molecule as such but rather for a peptide bound to allogeneic MHC (Matzinger and Bevan, 1977), and there are some data that show this might be the case (Marrack and Kappler, 1988; P.Panina unpublished results).

The model discussed so far deals with the capture, processing and association with class II of soluble antigens. It has been known for a long time that such exogenous antigens are not recognized in association with class I molecules [which are however very efficient at presenting endogenous viral antigens to the cytotoxic T lymphocyte (CTL)]. This surprising finding has not received any satisfactory explanation so far. Indeed we know that (i) there are class I determinants in soluble proteins, since these proteins can be recognized with class I, when engineered into a virus and (ii) there are sites available for binding immunogenic peptides on surface class I molecules (Townsend et al., 1986). How can the peptides of exogenous processed antigens avoid being associated with class I? It seems unlikely that class I determinants are not generated upon processing of exogenous antigens. In addition, our data demonstrate that class I is not functionally segregated from the processing pathway, since anti-class I antibodies can be efficiently processed and presented with class II. A possibility that would be worth testing is that in the endosomes, perhaps because of the low pH, class I molecules might not have a favourable conformation for binding peptides.

Although the mechanism for the class II/class I discrimination is purely speculative, and its existence has been challenged by some recently reported exceptions (Staerz et al., 1987; Yewdell et al., 1988; De Libero et al., 1988), one can see strong selective pressures not to have exogenous proteins presented with class I. In fact, such presentation would result in killing of the APC by a CTL, an event that would severely damage the organism since class I is present on all nucleated cells.

The growing family of APC

Once we accept that class II is required for presentation of exogenous antigens, it is natural to ask whether any class II positive cell can perform this task. In asking this question one should bear in mind that, since T cells recognize a complex of antigen and class II, the T cell response will be dependent not only on the concentration of processed antigen, but also on the concentration of class II molecules and that this concentration may vary in different cell types and is influenced by antigenic stimulation (Matis et al., 1983; Unanue et al., 1984).

We have examined the case of activated human T cells which, in spite of the high expression of class II molecules, have been repeatedly reported to be unable to present soluble antigens. We found that indeed T cells are not capable of presenting soluble antigens even when given at high concentrations, but they are very efficient at presenting antigens that specifically bind to their surface molecules. In fact mAbs against T-cell surface determinants, as well as the gp120 glycoprotein of HIV (which binds to CD4), can be processed and presented by T cells to specific T cells (Lanzavecchia et al., 1988b).

These results suggest that T cells are fully capable of processing and displaying antigens and are mainly limited in antigen presentation by their inefficiency at antigen capture. In vivo the antigen presenting capacity of T cells will therefore be insignificant in most cases, because very few antigens will bind directly to T cells, but in some instances, such as the case of HIV gp120, the situation might change dramatically and result in a T−T interaction that might have immunopathological consequences.

How big will the APC family grow? Class II is expressed not only on cells of the immune system, but also on epithelial cells at critical anatomical sites, such as the mucosae, where antigens are encountered. If these cells are capable of capturing antigens efficiently, there are no a priori reasons why they should not be the cells of choice for presenting environmental antigens.

Since class II expression on many cells can be upregulated by T cell products, one can envisage this as a means of increasing the number of APCs for protective immunity (Unanue et al., 1984). The same mechanism, however, might have dangerous consequences if the cell which is upregulating class II also efficiently picks up and processes a self antigen. For instance in auto-immune thyroiditis, thyrocytes express abnormally high levels of class II molecules, suggesting that they might present self antigens to autoreactive T cells (Hanafusa et al., 1983). Since a specific function of thyrocytes is to pick up and degrade thyroglobulin, they might well be capable of presenting this self antigen with high efficiency.

In conclusion class II-restricted presentation is a finely tuned function and most of the regulation is at the level of either antigen capture or class II expression, while processing seems not to be, at least in the cases examined, a major limiting factor. Although there is evidence for epitope-specific defects of processing in different individuals (Corradin et al., unpublished results), it seems likely that the antigen processing compartment might be present in all cells and carried out by the same machinery which is involved in the physiological turnover of cellular proteins.

What is the relationship between T cell specificity and function?

So far I have discussed a series of events that lead to the display on APCs of the antigen in a form recognizable by

T cells. I want to outline now what might be the functional consequences of this specific recognition.

The function of the immune system is to recognize foreign structures and trigger a series of reactions that eventually lead to their elimination. Effector function has therefore to be tightly linked to specific recognition in order to be focused on the specific target and to avoid damage to other cells. Antibodies achieve this coupling by having the two functions performed by distinct domains of the same molecule. Thus, both specificity and effector function are constitutive properties of one single immunoglobulin.

T cell-mediated responses are more complex. Specific recognition and effector function occur at the level of a transient contact between a helper cell (Th) and an APC or between a cytotoxic T lymphocyte (CTL) and its target. In recent years it has become evident that many of the effector functions of T cells are mediated by non-specific soluble factors. T cells synthesize and secrete a number of lymphokines which can exert different functions on the B cells (reviewed by O'Garra et al., 1988). CTL also produce and secrete lymphokines and, in addition, release pore-forming molecules (perforins) which can directly damage the target cell membrane (Dennert and Podack, 1983;

Lanzavecchia and Staerz, 1987). All these non antigen-specific mediators act only over a very short range or by cell–cell contact and are obviously most effective when a T lymphocyte is juxtaposed with an interacting cell.

Several questions arise in trying to understand the relationship between specificity and function of T cells: (i) Is the T cell effector function constitutive or has it to be induced? (ii) What is the role of the TCR? Is it required to provide specific contact, to trigger effector function, or to deliver it? (iii) How can the effector function be precisely directed to a given target cell?

To distinguish different epochs in the life of a T cell I will use the term 'virgin' to indicate mature peripheral T cells that have never seen antigen, 'activated' to indicate a T cell whose TCR has been previously engaged in antigen recognition and consequently has entered the growth/maturation program (a state that can be indefinitely maintained in T cell clones by adding exogenous IL-2) and 'triggered' to indicate an activated T cell with its TCR actually engaged in antigen recognition and therefore performing its effector function. These definitions are not purely academic, since T cell proliferation, although crucial for clonal expansion, is not necessarily associated with helper
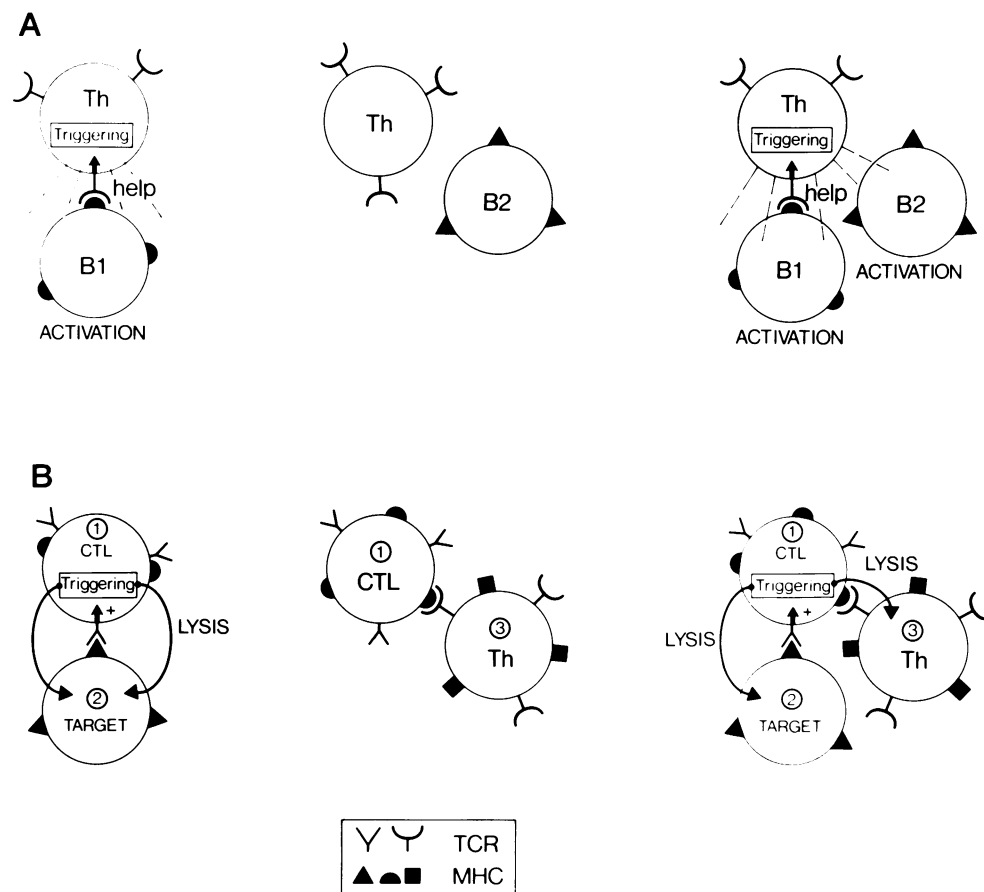

Fig. 2. The TCR is required for triggering the T cell effector function, but not for its delivery to the target cell. (A) Cognate interaction and bystander help. (i) Cognate interaction: A Th recognizes an alloantigen on a B cell (B1), is triggered through the TCR and stimulates the B cell to growth and maturation. (ii) No help is delivered to a B cell (B2) which is not recognized by the TCR of the Th. (iii) Bystander help. When the three cells are close together the Th is triggered by recognizing the antigen on B1 and help is delivered to both B1 and B2. (B) Direct and backwards killing: (i) Direct killing. A CTL (1) binds to the specific target (2). The occupation of the TCR triggers effector function and the target 2 is lysed. (ii) A CTL (1) is recognized by a Th clone (3) that binds to it. Since the TCR of the CTL is not occupied, the CTL is not triggered and cell 3 is not lysed. (iii) Backwards killing: when the two interactions occur simultaneously the CTL is triggered by recognizing cell 2 via the TCR and the lytic function is exerted on both cell 2 and (although with lower efficiency) cell 3.

or killing activities, i.e. an activated cell is not always a triggered cell.

Two *in vitro* models involving both T helper and CTL allow the exploration of the role of the TCR in delivering the effector function. A first example comes from studies with T helper clones specific for allogeneic class II molecules (Figure 2a). An alloreactive Th clone, by definition an activated T cell, does not constitutively provide help to autologous B cells. However, when the Th clone is triggered through its TCR by recognition of the specific alloantigen, help for B cells is generated. In this case, not only allogeneic B cells (which are recognized by the TCR), but also autologous B cells (which are not recognized) can be stimulated to grow and mature with similarly high efficiency. Since the autologous B cells are not specifically interacting with the Th cells, this form of help can be defined 'bystander help' (Lanzavecchia, 1983; De Franco *et al.*, 1984). Another example of bystander help comes from *in vitro* studies on the requirements for activation of cytotoxic precursors (Ramarli *et al.*, 1984).

Since killing requires direct cell contact, to study the role of the TCR in T cell killing I had to use the system of interacting cell clones shown in Figure 2b (Lanzavecchia, 1986). This system consists of a CTL clone (cell 1) that can recognize and kill a specific target (cell 2), and includes another cell (cell 3), which specifically recognizes and binds to the CTL clone, but is not recognized by it. The point of the experiment is to ask what will be the fate of cell 3 when binding to the CTL.

The results show that cell 3, which binds to an activated CTL, is not killed. However, when cell 2 is added, thereby providing the specific ligand for the TCR of the CTL, the CTL is triggered to perform its cytotoxic function. This results in killing not only of the specific target (cell 2) but also (although with a 5- to 10-fold lower efficiency) of cell 3. Therefore, cell 3 is killed when it binds to a triggered, but not to an activated, CTL. Since killing in this case goes in the opposite direction of recognition, I call this form of killing 'backwards killing'.

These experiments dissociate the triggering step from the delivery of the lethal hit and indicate that: (i) activated CTL are not constitutively cytotoxic but become cytotoxic only when triggered through the TCR and (ii) the cytotoxic activity is not delivered directly by the TCR and in this sense is not antigen-specific.

The example of backwards killing carries many analogies with that of bystander help. Indeed, in both cases the effector function is triggered by antigen through the TCR but is antigen non-specific, since it can be directed to cells which are not recognized by the TCR itself. Thus in T lymphocytes specificity and function are not physically linked (as they are in the antibody molecule) but only functionally associated by the triggering of the TCR. Potentially this poses the problem of locally indiscriminate help or killing.

In vitro experiments must be carefully interpreted since they often represent conditions that can only exceptionally occur *in vivo*. Indeed, in apparent contrast with what has been described so far, *in vivo* experiments have shown that in most cases cytotoxic and helper activities are precisely focused on the cells that bear the specific antigen (see, for CTL, Lukacher *et al.*, 1984). How can a non-specific effector mechanism be targeted so precisely? There are two separate requirements for the induction of help or

cytotoxicity: (i) occupancy of the TCR leading to prompt but transient secretion of mediators and (ii) contact or proximity between the interacting cells. Although these requirements can be experimentally distinguished, they are usually simultaneously satisfied by the bridging and triggering property of the TCR. Thus, the non-specific signal has a much higher chance of hitting the specific target than any other cell that might fortuitously come into contact with the effector T cell. In addition, it has been recently shown that T cells interacting with their specific target become transiently polarized towards the site where the TCR is engaged in antigen recognition (Kupfer *et al.*, 1986) and that lymphokines are preferentially released at the site where the TCR is triggered (Poo *et al.*, 1988). Although this directional exocytosis is not absolute and is not found at high levels of stimulation, it may certainly increase the chances that a T cell hits the right target.

In conclusion lymphokines and perforins resemble neurotransmitters rather than hormones in that they are effective mainly at the level of a mobile Th−APC or CTL−target interaction which can be envisaged as an immunological synapse.

What else besides the antigen?

The experiments described above addressed the questions of how antigens are displayed on APCs and how their recognition by the TCR leads T cells to effector function.

A large body of information has accumulated in the last few years on these two subjects. On the one hand, there is the crystal structure of a class I molecule and there are models to predict how immunogenic peptides can bind to class II and be seen by the TCR (Björkman *et al.*, 1987; Brown *et al.*, 1988; Davis and Björkman, 1988). On the other hand, many lymphokines have been cloned and their functions and receptors characterized (for recent review see O'Garra *et al.*, 1988).

Among the many open questions that still remain, the most challenging for me is to understand what other contacts or signals, in addition to the specific interaction between TCR and antigen−MHC, may be required for effective communication at the level of the immune synapse.

This issue is complicated by the fact that APCs are heterogeneous and T cells at different stages in their life might have different requirements for activation and triggering, or may simply respond differently to the antigenic stimulation. It has been demonstrated that distinct antigen non-specific contacts may be required for T cell activation, but their requirement is never absolute. In addition it is not always clear whether these accessory contacts simply increase the avidity of the cell−cell interaction or transduce signals. Last, but not least, is the question of whether the APC is just passively displaying antigens and accessory signals or whether it has to be activated by the T cell in order to become stimulatory.

Understanding the rules of cellular communication at the level of the immune synapse will help to elucidate the relationship between different cell types in the generation of the mature T cell repertoire and in the induction and regulation of the immune response.

At the end of these pages I realize also how important it was for me to be in the right place, not only in terms of facilities to work and freedom to follow my research interest, but also in terms of interaction with a number of colleagues

with different backgrounds and complementary interests. A place where ideas can be discussed, criticized and challenged at the very same time they are conceived. Since 1983 I have found all this at the Basel Institute for Immunology.

I wish to thank all my colleagues at the Basel Institute for many stimulating discussions, Gennaro De Libero, Luciana Forni, Uwe Staerz, Charles Steinberg, Gitta Stockinger and Colin Watts for reading this manuscript and Janette Millar for typing it.

References

Babbitt,B.P., Allen,P.M., Matsueda,B., Haber,E. and Unanue,E.R. (1985) *Nature*, **317**, 359–361.

Björkman,P.T. *et al.* (1987) *Nature*, **329**, 506–512.

Bottazo,G.F., Pujol-Borrell,R., Hanafusa,T. and Feldmann,M. (1983) *Lancet*, **ii**, 1115–1118.

Brown,J.H., Jardetzky,T., Saper,M.A., Samraoui,B., Bjorkman,P.J. and Wiley,D.C. (1988) *Nature*, **332**, 845–850.

Buus,S., Sette,A., Colon,S.M., Jervis,D.M. and Grey,H. (1986) *Cell*, **47**, 1071–1077.

Buus,S., Sette,A. and Grey,H.M. (1987) *Immunol. Rev.*, **98**, 115–142.

Cresswell,P. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 8188–8192.

Davis,M.M. and Björkman,P. (1988) *Nature*, **334**, 395–402.

De Franco,A., Ashwell,J.P., Schwartz,R.H. and Paul,W.E. (1984) *J. Exp. Med.*, **159**, 861–880.

De Libero,G., Flesch,I. and Kaufmann,S.H.E. (1988) *Eur. J. Immunol.*, **18**, 59–66.

Dennert,G. and Podack,E.R. (1983) *J. Exp. Med.*, **157**, 1483–1495.

Falo,L.D., Benacerraf,B. and Rock,K. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 6994–6997.

Kakiuchi,T., Chestnut,R.W. and Grey,H.M. (1983) *J. Immunol.*, **131**, 109–114.

Katz,D.H., Hamaoka,M.E. and Benacerraf,B. (1973) *Proc. Natl. Acad. Sci. USA*, **70**, 2624–2628.

Kupfer,A., Singer,S.J. and Dennert,G. (1986) *J. Exp. Med.*, **163**, 489–498.

Kupfer,A., Swain,S.J., Janeway,C.A. and Singer,S.J. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 6080–6083.

Lakey,E.K., Margoliash,E. and Pierce,S.K. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 1659–1663.

Lanzavecchia,A. (1983) *Eur. J. Immunol.*, **13**, 820–824.

Lanzavecchia,A. (1985) *Nature*, **314**, 537–539.

Lanzavecchia,A. (1986) *Nature*, **319**, 778–780.

Lanzavecchia,A. (1987) *Immunol. Rev.*, **99**, 39–51.

Lanzavecchia,A., Parodi,P. and Celada,F. (1983) *Eur. J. Immunol.*, **13**, 733–738.

Lanzavecchia,A. and Staerz,U.D. (1987) *Eur. J. Immunol.*, **17**, 1073–1074.

Lanzavecchia,A., Abrignami,S., Scheidegger,D., Obrist,R., Dörken,B. and Moldenhauer,G. (1988a) *J. Exp. Med.*, **167**, 345–352.

Lanzavecchia,A., Roosnek,E., Gregory,T., Berman,P. and Abrignani,S. (1988b) *Nature*, **334**, 530–532.

Lassila,O., Vainio,O. and Matzinger,P. (1988) *Nature*, **334**, 253–255.

Lukacher,A., Braciale,V.L. and Braciale,T.J. (1984) *J. Exp. Med.*, **160**, 814–826.

Manca,F., Kunkl,A., Fenoglio,D., Fowler,A., Sercarz,E. and Celada,F. (1985) *Eur. J. Immunol.*, **15**, 345–350.

Manca,F., Fenoglio,D., Kunkl,A., Cambiaggi,C., Sasso,M. and Celada,F. (1988) *J. Immunol.*, **140**, 2893–2898.

Matis,L.A., Glincher,L.H., Paul,W.E. and Schwartz,R.H. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 6019–6023.

Marrack,P. and Kappler,J. (1988) *Nature*, **332**, 840–843.

Matzinger,P. and Bevan,M.J. (1977) *Cell. Immunol.*, **29**, 1–5.

Mitchison,N.A. (1971) *Eur. J. Immunol.*, **1**, 18–27.

O'Garra,A., Uruland,S., De France,T. and Christiansen,J. (1988) *Immunol. Today*, **9**, 45–54.

Ozaki,S. and Berzofski,J.A. (1987) *J. Immunol.*, **138**, 4133–4142.

Pernis,B. (1985) *Immunol. Today*, **6**, 45–49.

Poo,W.J., Canrad,L. and Janeway,C.A. (1988) *Nature*, **332**, 378–380.

Ramarli,D., Parodi,B., Fabbi,M., Corte,G. and Lanzavecchia,A. (1984) *J. Exp. Med.*, **159**, 318–323.

Rock,K.L., Benacerraf,B. and Abbas,A.K. (1984) *J. Exp. Med.*, **160**, 1102–1113.

Roosnek,E., Demotz,S., Corradin,G.P. and Lanzavecchia,A. (1988) *J. Immunol.*, **140**, 4079–4082.

Rosenthal,A.S. and Schevach,E.M. (1973) *J. Exp. Med.*, **138**, 1194–1212.

Snider,M.D. and Rogers,O.C. (1985) *J. Cell. Biol.*, **100**, 826–834.

Staerz,U.D., Karasuyama,H. and Garner,A.E. (1987) *Nature*, **329**, 449–451.

Tony,H.P., Phillips,N.E. and Parker,D.C. (1985) *J. Exp. Med.*, **162**, 1695–1708.

Townsend,A.R.M., Rothbard,J., Gotch,F.M, Bahadur,G., Wraith,D. and McMichael,A.J. (1986) *Cell*, **44**, 959–968.

Uhr,J.W. and Möller,G. (1968) *Adv. Immunol.*, **8**, 81–126.

Unanue,E.R., Beller,D.I., Lu,C.Y. and Allen,P.M. (1984) *J. Immunol.*, **132**, 1–5.

Watts,C. and Davidson,H. (1988) *EMBO J.*, **7**, 1937–1945.

Yewdell,J.W., Bennick,J.R. and Hosaka,Y. (1988) *Science*, **239**, 637–690.