LECTURE

Time for integration: communication in the immune system

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Abstract. Signals exchanged between cells in the immune system are generated at specific contact regions termed "immunological synapses" (*IS*). The morphology and the functions of *IS* vary with circumstances and include directing secretion and integrating positive and negative signals to tune the extent of response – a model that is essentially derived from neurosciences. Indeed, the mechanisms of signal integration and antigen decoding are not yet completely understood and many hypotheses are worked out. (www.actabiomedica.it)

Key words: immunological synapse, intracellular signalling, signal integration, cytokine secretion

Introduction

Immune cells are migratory cells that move from one tissue to another and within each tissue. The immunological activation of defense functions is a complex, multistep process largely driven by direct or soluble factors-mediated communications between immune cells themselves, between immune cells and endothelia and between immune cells and pathogens or transformed cells. A correct communication is the essential component of the functional integration in the immune system. Of course, as the immune functions are flexible, communication among immune cells must be flexible: morphological stability is not required here.

Immunological synapses

The immunological synapses (*IS*) were originally described as junctions between T cells and antigen-presenting cells (APC) (1, 2) therefore subordinate to the T cell-APC adhesion (3, 4). Any T cell-APC or target-cell interface involved in information transfer could be described as *IS* (5). Observation of protein segregation at contacts between Natural Killer (NK) cells and target cells or B cells, extended the concept

of *IS* to these immunological effectors, as well. The *IS* structure can be stable for several hours and, although its organization shows variations related to the players involved (3, 6, 7), its formation is a process characterized by two main phases, defined on a morphological and molecular basis: i) *immature IS*, presenting an early, non-definitive protein organization; ii) *mature IS*, that develop in the order of minutes, with a protein arrangement consisting of a central and a peripheral supramolecular activation cluster (c-SMAC and p-SMAC, respectively) (see for extensive review 8).

T-helper cells form immature *IS* in which talin and leukocyte function-associated molecule-1 (LFA-1) are clustered in the c-SMAC, while T-cell receptor (TCR) localizes in the p-SMAC. The maturation of the synapse is characterized by the inversion of this arrangement: the mature *IS* presents talin and LFA-1 at the periphery with the TCR now localized in the c-SMAC (3, 4). It is not yet clear the mechanism by which in the *IS* the interaction of TCR with the major histocompatibility complex (MHC) is stabilized. It is supposed that the stabilization process involves a TCR-MHC-peptide-dependent oligomer formation (9) or an irreversible refolding process, which could be related to the self-catalyzed protein refolding in other systems (10).

Cytotoxic T-cells or lymphocytes (CTL) form immature *IS* characterized by TCR clusters surrounded by LFA-1 (11). Maturation of the *IS* leads to a c-SMAC with two segregated domains, TCR and lytic granules (12, 13). NK-cells initially form *IS* by SH2-domain-containing phosphotyrosine phosphatase-1 (SHP-1) clustered in small areas surrounded by LFA-1 (13, 14). Cytoskeletal rearrangement conveys the lytic granules in the c-SMAC surrounded by LFA-1 (14, 15).

The role of *IS* is not yet clear in all details. For sure *IS* represents the origin of intracellular signals that each cell integrates giving rise to an appropriate functional response. The *IS* maturation itself should be considered as an evolutive process in which the different steps can mediate different signals. For instance, a mature *IS* is not required to initiate T-cell activation (16). Moreover, the cytoskeleton involvement in the effects downstream *IS* formation is different in NK cells and CTL: a mild perturbation of cytoskeletal rearrangement can block cytolytic activity in NK cells but not in CTL. This has been related to the different regulation of cytotoxicity in the two cell types, i.e. more stringent by univocal TCR recognition in CTL, delicately tuned by a balance of activatory and inhibitory signals in NK cells (17). One common function of *IS* however seems to be directing the secretion of cytokines or lytic granules, which require mature *IS* (13, 18, 19). Among the different immunological effectors, T cells offer a more complete picture. The protein accumulation at the *IS* can increase the rate of TCR triggering, potentiating signalling (20). The fact that TCR ligation likely contributes to signalling while undergoing internalization is suggested by the observation that TCR stimulation leads to increased surface transport of TCRs, effectively supplying the cell with more receptors to facilitate sustained signalling (21). At the initial binding of TCR cognate peptide-MHC ligands, 1-20 engaged TCRs (22) are sufficient to start signalling, consisting in local tyrosine phosphorylation and in intracellular Ca^{2+} elevation (16). The downstream signalling cascade activates in sequence phosphatidylinositol 3-kinase (PI3K), Rho GTPases (23, 24), protein kinase B/Akt (23, 25). The persistent TCR signalling induces the accumulation of surface receptors and other signalling molecules, such

as LFA-1. Other receptors move within the lipid rafts, that form the platforms for the assembly of the signalling complexes (26). CD4, CD28, CTLA-4, CD9, tyrosine kinase Lck and the adaptor protein linker for activated T cells (LAT), that are all molecules providing costimulation and signalling integration, were found associated to the raft at the *IS* (27, 28).

In the cSMAC of maturing *IS*, TCR colocalize with the isoform θ of the protein kinase C (PKCθ), wich cooperates with the phosphatidylinositol-3-kinase (P13K) and CD28 to reinforce and prolong TCR signalling (3, 23, 29). TCR clustering promotes a process of "inside-out" signalling to LFA-1, that requires adaptor proteins, such as ADAP and SKAP-55 (30), and actin dynamics (31), ultimately leading to the segregation of LFA-1 in the pSMAC, where it remains connected to the cortical actin cytoskeleton by talin (30) (Fig. 1).

Signal integration

T-cell activation requires a prolonged intracellular signaling over several hours (32); however, the signal emanating from each single TCR is short-lived, so it is necessary a countinuous TCR triggering to sustain signalling, which would otherwise drop down (33, 34). Around this concept the current theory supports the idea that small and short signals that alone are unable to trigger a T-cell response are summed up over time to reach the threshold level, with a higher efficiency if the triggered receptors are in close proximity. Low affinity ligands would therefore enhance T-cell responses induced by agonists adding their transient signals to the main one. Consequently, the extent of temporal summation in the presence of low affinity ligands is higher than that achieved by agonist alone (35). Moreover, the costimulation seems to play a central role in the stabilization of TCR-induced tyrosine phosphorilation (36), acting as an amplifier at the *IS*, where TCR and ligands are packed in a limited space (37, 38).

The concept of *IS* as the molecular and morphological basis of communication in the immune system provides a model that has analogies with − and is, after all, derived from − neurosciences. At least for T-cells − where the model is better understood − signal
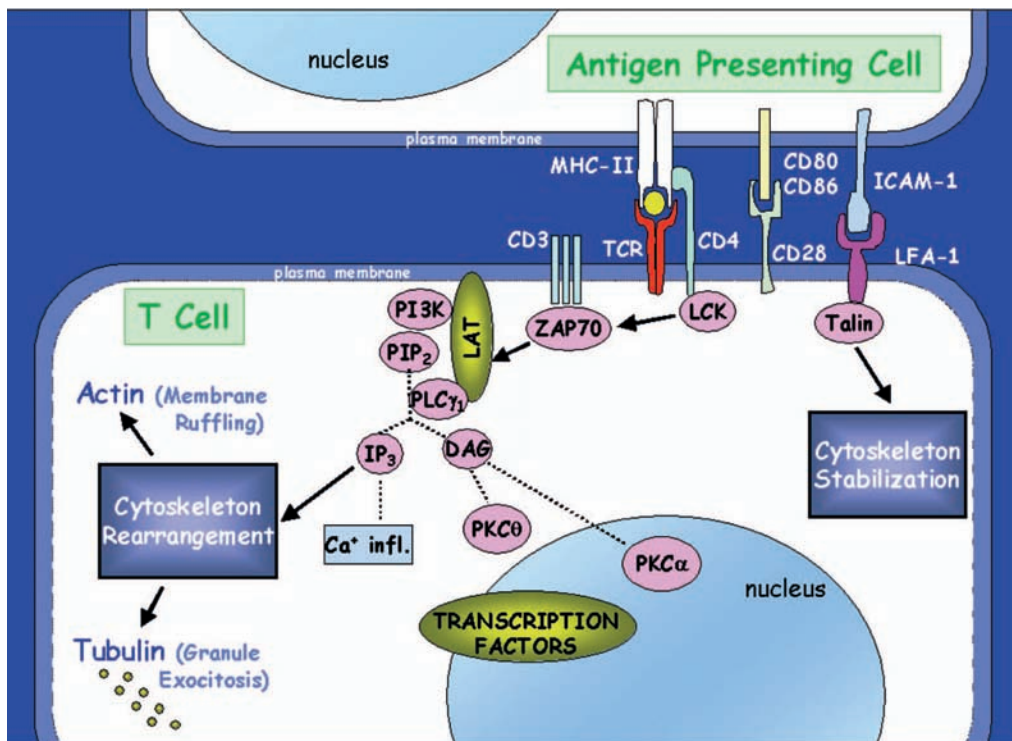
Figure 1. Schematic diagram of the fundamental molecular interactions and intracellular signalling pathways that play a role upon T helper cell recognition of Antigen Presenting Cells

summation and integration at the single cell level determines the type and extent of cellular response. Most experimental immunology has been devoted to the dissection of specific receptor-ligand interactions and their down-stream signalling, and relevant advances have been achieved in these last decades by this methodological approach, separating the odds and ends. Neurosciences teach immunologists now that, with few exceptions, single interactions and their down-stream signalling essentially describe *in vitro* situations that may not resemble the real cell regulation systems that are rather the integrated product of a series of signals from different sources that are brought to the cell in that specific period of time in that specific anatomical/molecular environment.

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