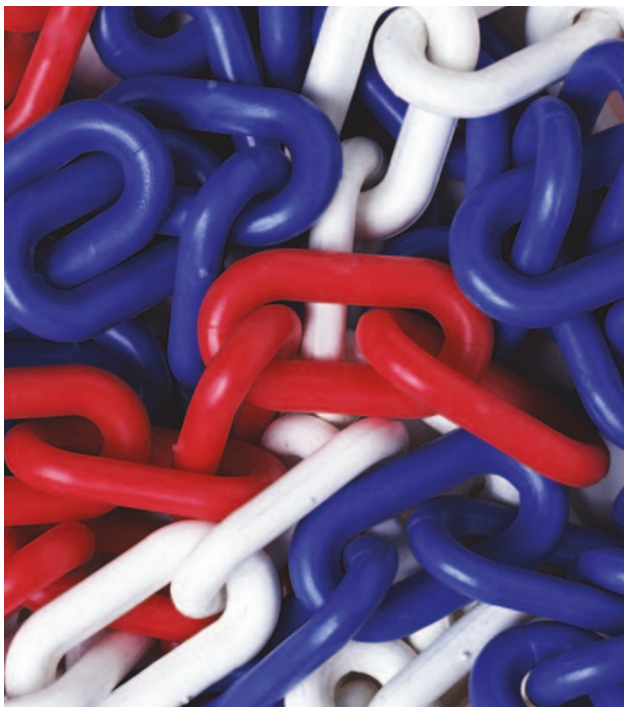
AUTOIMMUNITY

Infection and autoimmunity: the glycolipid link



Although the association between bacterial infection and autoimmune disease is well known, there is little evidence for a specific mechanistic link. One hypothesis — the antigen non-specific theory — proposes that the widespread cellular damage that is inflicted by microbial infection might expose hidden self-antigens to autoreactive T cells. Now, reporting in *Immunity*, Gennaro De Libero and colleagues propose a more refined model, in which bacterial infection induces the production of large amounts of endogenous glycosphingolipid (GSL) antigen by host cells. The authors suggest that the recognition of these GSLs by 'self-directed' T cells might contribute to autoimmune disease.

De Libero and co-workers focused their studies on T-cell clones that had been derived from patients with multiple sclerosis; these cells are specific for GSLs that are abundantly expressed in the central nervous system. Because GSLs are presented to T cells by CD1 molecules — MHC-class-I-like molecules that are implicated both in the activation of pro-inflammatory T-cell responses to mycobacterial products and in the generation of autoreactive lymphocytes — the authors reasoned that further analysis of the CD1–T-cell interaction might reveal the mechanisms that link infection and autoimmunity.

Indeed, the self-GSL-specific T cells were activated by antigen-presenting cells (APCs) that had been either infected with bacteria or exposed to microbial products such as lipopolysaccharide (LPS). T-cell stimulation required ligation of the T-cell receptor by the CD1 complex, indicating an antigen-specific activation process. This led the authors to speculate that increased synthesis of endogenous GSLs might be the trigger that induces the autoreactive T-cell response. Further biochemical analysis proved that this was the case: bacterially infected APCs or APCs that had been treated with LPS upregulated the synthesis of GSLs, and the authors propose that the increased numbers of GSL–CD1 complexes at the surface of APCs activate the self-GSL-specific T cells.

These studies provide insights into the microorganism-induced

IMMUNOLOGICAL SYNAPSES

No rafts required

The T-cell–antigen-presenting-cell contact site is a highly organized complex of cell-surface receptors and associated intracellular-signalling proteins that is known as the immunological synapse. Although the components of the immunological synapse are well defined, the mechanisms by which it forms are poorly understood. Now, a report published in *Cell* indicates that protein–protein interactions can direct the formation of discrete microdomains of receptors and signalling molecules after T-cell activation.

Previous studies have implicated both lipid rafts and actin and myosin cytoskeletal networks as mediators of protein clustering at the immunological synapse. So, to further investigate the mechanisms by which microdomains of signalling molecules form, Douglass and Vale used both conventional confocal microscopy and single-molecule tracking to analyse the overall membrane distribution and single-molecule behaviour of green fluorescent protein (GFP)-tagged T-cell-signalling molecules, after the activation of Jurkat T cells by immobilized T-cell receptor (TCR)-specific antibodies.

In the absence of T-cell activation, individual molecules sometimes moved rapidly and at other times were static. However, the different signalling molecules showed distinct patterns of mobility: CD2 molecules were mostly immobile, with only occasional periods of rapid mobility, whereas both a marker of lipid rafts (the ten amino-terminal amino acids of LCK (LCK10) fused to GFP) and a non-raft-associated protein (CD45) spent little time immobile. These patterns of diffusion indicate that immobilization was not due to lipid-raft formation. Consistent with this, after TCR crosslinking, both raft-associated wild-type LAT (linker for activation of T cells) and a LAT mutant that cannot associate with lipid rafts but can mediate protein–protein interactions (LAT(C-S)) showed reduced mobility.

After TCR crosslinking, CD2 molecules were observed to cluster and to colocalize with LCK and LAT but not CD45. Colocalization of LCK and CD2 was not a result of LCK being a raft-associated protein, because the lipid-raft marker LCK10 did not colocalize with CD2. Furthermore, a raft-associated LAT mutant that cannot be phosphorylated (LAT(Y-F)) did not colocalize with CD2, indicating that protein–protein interactions mediated by tyrosine-phosphorylated LAT mediate colocalization of LAT and CD2. Consistent with this, CD2 clustering after TCR crosslinking was impaired in a cell line derived from Jurkat T cells that has severely reduced LAT expression, and this clustering could be restored by transfecting the cells with LAT but not LAT(Y-F).

Further single-molecule analysis indicated that CD45 was excluded from CD2-containing microdomains, whereas LCK, LAT and LAT(C-S) were often associated with these microdomains and, when associated, showed little mobility. Together, these studies indicate that LCK and LAT are preferentially trapped in the CD2-containing microdomains through protein–protein interactions and that the association of these proteins with lipid rafts does not regulate this process. This led the authors to suggest that protein–protein interactions should be considered to be an early event in T-cell signalling and a mechanism of immunological-synapse formation.

Karen Honey

References and links

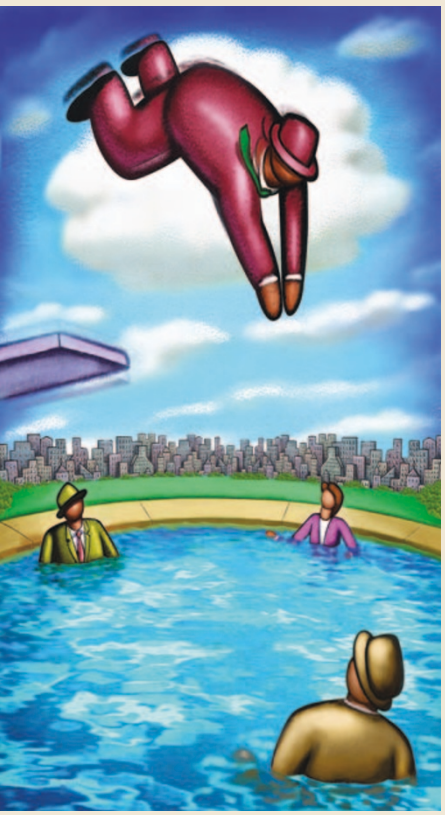
ORIGINAL RESEARCH PAPER Douglass, A. D. & Vale, R. D. Single-molecule microscopy reveals plasma membrane microdomains created by protein–protein networks that exclude or trap signaling molecules in T cells. *Cell* **121**, 937–950 (2005)

pathways that upregulate GSL synthesis. Because different bacteria and bacterial products activated GSL-specific T cells, it is probable that several different pattern-recognition receptors and signalling pathways converge on the GSL biosynthesis machinery. Notably, only intact LPS could prime the APCs for optimal T-cell activation; lipid A or modified LPS was ineffective in this respect.

With regard to the physiological functions of GSL-specific T cells, these remain contentious, but because these cells respond to APCs that are primed by microbial infection, it is probable that they have a role in either promoting or modulating the initial immune response to infection. After resolution of infection, these self-GSL-specific T cells are a repository of potentially autoreactive lymphocytes.

Shannon Amoils, Assistant editor,
Nature Reviews Microbiology

References and links
ORIGINAL RESEARCH PAPER De Libero, G. *et al.* Bacterial infections promote T cell recognition of self-glycolipids. *Immunity* **22**, 763–772 (2005)
FURTHER READING Kronenberg, M. and Kinjo, Y. Infection, autoimmunity, and glycolipids: T cells detect microbes through self-recognition. *Immunity* **22**, 657–659 (2005)



LYMPHOCYTE RESPONSES

How RAG2 cycles

The generation of lymphocyte-specific antigen receptors by V(D)J recombination is controlled by levels of recombination-activating gene 2 (RAG2) protein, which accumulates during the G1 (gap 1) phase of the cell cycle but is rapidly degraded at the G1–S (synthesis)-phase transition, continuing until the next entry to G1. This ensures that the double-stranded DNA breaks that are created by RAG2 can be repaired correctly by non-homologous end joining (NHEJ), which is also most active during G1, and this might prevent genomic instability. A recent study in *Molecular Cell* has determined the molecular mechanisms that link RAG2 degradation to the cell cycle.

The authors used a cell-free *in vitro* system consisting of an extract of HeLa cells synchronized in S phase to which RAG2 constructs — in the form of a glutathione *S*-transferase fusion protein containing the carboxy-terminal 89 amino-acid residues of RAG2 (denoted GST–R2CT89) — were added. To confirm that this system recapitulates the *in vivo* situation, they showed that a mutation of the threonine residue at position 490 of RAG2 that inhibits degradation *in vivo* also reduced the degradation of RAG2 (in the form of GST–R2CT89(Thr490Ala)) in the *in vitro* system.

In the cell-free S-phase system, addition of a proteasome inhibitor or ubiquitin mutants that inhibit ubiquitin branching impaired GST–R2CT89 degradation, and the involvement of the ubiquitin–proteasome pathway in RAG2 degradation was then confirmed in S-phase cells. By contrast, the levels of GST–R2CT89(Thr490Ala) were not affected by proteasome inhibition. Previous genetic evidence has indicated that the cyclin-dependent kinase (CDK) inhibitor p21 and the cyclin-A–CDK2 complex are negative and positive regulators, respectively, of this RAG2-degradation pathway. Indeed, in the cell-free system, addition of p21 to, or depletion of CDK2 or cyclin A from, the S-phase extract inhibited the ubiquitylation and degradation of GST–R2CT89.

The substrate selectivity of ubiquitylation is mainly determined by E3 ligases, so the authors next looked for an E3 ligase that recognizes the carboxyl terminus of RAG2 in fractions of S-phase extract. One fraction contained substantial ubiquitylation activity against GST–R2CT89, and this fraction was enriched for SKP2, which is a subunit of the SCF-family multicomponent E3 ligases. Depletion of SKP2 from the S-phase extract markedly reduced ubiquitylation and degradation of GST–R2CT89.

SKP2 was shown to interact specifically with the Thr490-phosphorylated form of RAG2 but not with other phosphorylated or unphosphorylated forms. The low-degradation activity of a G1-phase extract could be restored by addition of cyclin A, CDK2 and SKP2, showing that these are the limiting factors for RAG2 degradation. The authors suggest a model in which RAG2 is phosphorylated at Thr490 by cyclin-A–CDK2, which creates a binding site for SKP2, leading to ubiquitylation and degradation. The involvement of SKP2 in the destruction of RAG2 was confirmed *in vivo*: RAG2 accumulation was uncoupled from the cell cycle in thymocytes from a SKP2-deficient mouse, and expression of a dominant-negative mutant of SKP2 in HeLa cells led to increased accumulation of RAG2 protein.

SKP2 is a central regulator of cell-cycle progression, so the finding that SKP2 is also involved in RAG2 degradation provides a direct mechanistic link between DNA recombination and the cell cycle. This ensures that RAG2 expression is restricted to G1 to coordinate the generation of RAG-induced DNA breaks with repair by NHEJ.

Kirsty Minton

References and links
ORIGINAL RESEARCH PAPER Jiang, H. *et al*. Ubiquitylation of RAG-2 by Skp2–SCF links destruction of the V(D)J recombinase to the cell cycle. *Mol. Cell* **18**, 699–709 (2005)