acetylases by $Stat1_{S727A}$ might be the mechanism responsible for the inability of this mutant Stat1 protein to activate transcription. If this hypothesis is true, one would predict that histone acetylation might be normal at the promoters of S727 phosphorylation-insensitive genes, such as SOCS1 and MHC I, in cells expressing $Stat1_{S727A}$; something still to be determined. Another important related issue is the role of CBP in Stat1 S727 phosphorylation-dependent gene activation. Because the $Stat1_{S727A}$ mutant protein was defective in ability to interact with CBP in cell extracts, S727 phosphorylation-dependent recruitment of CBP to gene promoters may not explain the differential gene enhancement by phosphorylation of Stat1 S727. One possibility is that histone acetylases other than CBP may be recruited to the promoters of those S727 phosphorylation-insensitive genes. Interestingly, the chromatin remodeling factor Brg1 (Brahma-related gene 1) has recently been shown to regulate only a subset of Stat2-dependent genes (Huang et al., 2002). It will be of great interest to know if a similar factor is involved in the selective regulation of S727 phosphorylation-dependent genes. The work by Varinou et al. has clarified the physiological role of Stat1 serine phosphorylation. At the same time, it has raised additional interesting and challenging questions for the JAK-STAT research field.

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Molecular Interactions: Stiff or Floppy (or Somewhere in Between?)

Recognition of MHC and MHC-like molecules by both natural killer (NK) and T cell receptors (TCR) reveals remarkable degeneracy. The interaction of the NKG2D NK receptor with several MHC I-like ligands has now been analyzed thermodynamically by McFarland and Strong, who suggest that a "rigid adaptation" mechanism governs such crossreactivity. This contrasts with "induced fit" that accounts for TCR adaptation to multiple MHCp ligands.

The best understood of the cell surface receptors that govern immune recognition by natural killer (NK) cells and T lymphocytes exploit either C-type lectin-like or immunoglobulin-like scaffolds to support structures that bind either MHC I-like molecules or classical MHC/peptide (MHCp) complexes. The interaction of NK receptors with MHC class I-peptide (MHCIp) or with MHC I-like molecules activates costimulatory or inhibitory pathways, depending on the particular NK receptor engaged and the physiological state of the effector cell (Raulet, 2003). Recent efforts to understand the interaction of NK receptors with MHC molecules have included X-ray structure determination of a number of NK receptor/MHC complexes and detailed studies of various NK receptors binding to their MHCIp or MHC I-like ligands (Natarajan et al., 2002). These studies parallel efforts to understand the molecular basis of T cell recognition based on crystal structures of TCR/MHC complexes and analysis of the kinetics and thermodynamics of these interactions (Rudolph et al., 2002).

Dilemmas have arisen in both the NK and TCR recognition arenas: how do some NK receptors manage to bind several different MHCp or MHCI-like molecules that only show some 20% identity in linear amino acid sequence? How do some TCR bind distinct MHCp complexes in which the bound peptides are quite different, or interact with MHC molecules of different alleles or species?

The latest chapter in this ongoing tale of the molecular basis of degenerate recognition by the C-type lectin like NK receptor, NKG2D, is reported in this issue of *Immunity* (McFarland and Strong, 2003). NKG2D is a homodimeric, type II membrane protein, characteristically expressed on NK cells, some NK T cells, $\gamma\delta$ T cells, resting $CD8^{+}\alpha\beta$T cells [this is true only for humans; mouse CD8 T cells must be activated before expressing NKG2D], and resting macrophages that are associated with a signal transducing subunit, either DAP10 or DAP12 (Raulet, 2003). Ligands of NKG2D, though somewhat different in the mouse and human, have several features in common: they reveal structural similarity to the classical $\alpha1\alpha2$ domain unit of classical MHCI molecules (though they lack bound peptide or a peptide binding groove), and they seem to be expressed in response to various kinds of cellular stress such as heat shock, tumor transformation, and bacterial or viral infection. In the human, NKG2D binds the peptide-free epithelial cell expressed MHCI-like molecules, MIC-A and MIC-B (Bauer et al., 1999). These are cell surface molecules

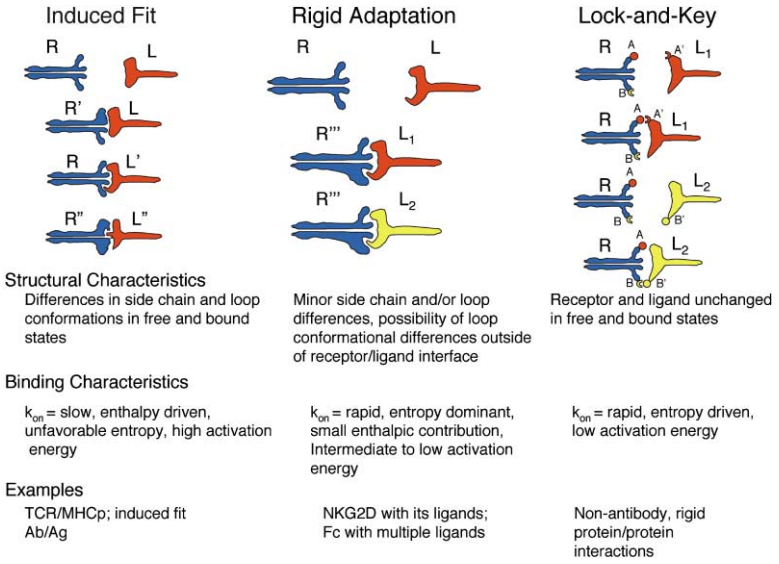

Figure 1. Schematic Illustration of Three Different Mechanisms for Receptor/Ligand Interactions

The binding surfaces of a receptor, R and its ligand, L, are illustrated for each of the three models, induced fit, rigid adaptation, and lock-and-key. Three different modes of induced fit are illustrated: one in which only the ligand, L, changes conformation on binding to L′; one in which the receptor itself, R′, changes on binding, and a third in which both receptor R″ and ligand L″ undergo conformational change. For rigid adaptation, modest conformation change outside of the binding site may occur for the receptor (R goes to R‴), but different ligands do not undergo conformational change. An example of lock-and-key recognition of degenerate ligands is shown where subsites A and B on the receptor and the respective subsites A′ on one ligand and B′ on a second ligand are shown. Structural and binding characteristics of the three modes of interaction and examples of each are tabulated.

with MHCI-like α1,α2, and α3 domains that lack the β2-microglobulin subunit characteristic of classical MHCI molecules. Significant amounts of soluble MIC molecules can be found in the serum of patients with epithelial tumors (Groh et al., 2002). Other NKG2D ligands of the human include distantly related MHCI-like molecules known as ULBP1, 2, and 3 that only have α1 and α2 domains and that are linked to the cell surface via a glycophosphatidyl-inositol (GPI) anchor, as well as the similarly sized and structured RAET1 molecules (including ULBP4 or RAET1E) that have genuine transmembrane domains. The human NKG2D seems to be universally associated with DAP10, a signal transducing molecule with a YXXM motif that mediates activation through a PI3 kinase pathway. The mouse NKG2D molecule also binds stress associated MHCI-like molecules, Rae-1, H-60, or MULT1. MuNKG2D is expressed as different isoforms that interact either with DAP10, as in the human, or with DAP12, allowing signaling through a ZAP70 or SYK pathway.

The X-ray structures of both murine (Wolan et al., 2001) and human (McFarland et al., 2003) NKG2D as well as complexes consisting of huNKG2D and MIC-A (Li et al., 2001), huNKG2D and ULBP3 (Radaev et al., 2001), and muNKG2D and Rae-1β (Li et al., 2002) have been solved and offer a tangible, static view of how a single receptor can interact with multiple ligands. The NKG2D homodimer uses each of its monomers to interact asymmetrically with either the α1 helix or the carboxyl half of the α2 helix of its MHCI-like ligands, in a general orientation similar to that of the TCR/MHCp interaction. The two identical subunits of the NKG2D homodimer use different residues to mediate interaction with each of the α helices of their ligands.. Furthermore, direct comparison of the interactions of huNKG2D with MIC-A and ULBP3 demonstrated that largely nonoverlapping subsets of contact residues are employed by the huNKG2D to bind the latter two molecules. Thus analysis of the three different receptor-ligand structures provides information on six different modes of NKG2D binding. Mutagenesis studies in two laboratories (McFarland et al., 2003; Radaev et al., 2002) confirmed that the NKG2D receptor utilizes different strategies to interact with each of its targets.

In structural terms, how can one explain degenerate recognition such as that seen with NKG2D? There are two extreme models (illustrated in Figure 1): (1) induced fit, in which the receptor adjusts conformationally to allow the formation of a stable complex; or (2) rigid lock-and-key, in which the receptor remains in precisely the same conformation before and after interaction with a rigid ligand, preserving strict shape and/or charge complementarity. For model 1, conformational adjustment of one or more loops and/or amino acid side chains is required to yield the match needed for a stable complex. Variations of model 1 include those in which the ligand changes its conformation, either with or without the adjustment of the receptor. For a rigid receptor to interact with multiple distinct ligands as in model 2, the partners must satisfy shape and charge complementarity requirements, although this may be accomplished in different ways. For example, if the receptor has binding subsites A and B, ligand 1 might have a complementary subsite A′, while ligand 2 could have a distinct complementary subsite B′. This is acceptable as long as other parts of the receptor-ligand interface are not disruptive or inhibitory to the interaction. Strictly speaking, comparison of the structures of the receptor and ligand in the free and bound states should be sufficient to substantiate either of the extreme models. Indeed, initial interpretation of the NKG2D/ULBP3 structure suggested an induced fit mechanism. More recently, however, with the availability of the unliganded huNKG2D structure, McFarland et al. (2003) favored a view in which NKG2D flexibility plays a minor role.

Resolution of a controversy often derives from a new avenue of experimentation, and for NKG2D the fruitful approach has been to examine the thermodynamics of the binding of muNKG2D with Rae-1β and of huNKG2D with MIC-A, MIC-B, and ULBP1. Early kinetic binding

studies of TCR/MHCp suggested slow association rate constants, a hint that conformational adjustment (or induced fit) might be needed (Corr et al., 1994). NKG2D/ligand complex formation was more rapid, consistent with rigid body interactions. A simple binding analysis would yield an equilibrium dissociation constant (K_D), and thus the change in free energy on binding given by $\Delta G^\circ = - \ln K_D$. Such an analysis would not in itself tell us anything about the mechanism of binding. However, by evaluation of the binding parameters as a function of temperature, following an approach recently applied to TCR/MHCp binding in two laboratories (Boniface et al., 1999; Willcox et al., 1999), one can estimate the heat capacity (ΔC°_P), the enthalpy (ΔH°, the heat change on binding), the entropy (ΔS°, the disorder that accompanies the reaction), and the activation energy that govern the interactions. Careful determination of these parameters for the four NKG2D interactions, huNKG2D with MIC-A, MIC-B, and ULBP1, and muNKG2D with RAE-1β, and comparison of these data to those obtained in similar analyses of TCR/MHCp interactions leads to the general finding that NKG2D-ligand interactions, which are of somewhat higher affinity than TCR/MHCp interactions, are governed by entropy rather than enthalpy. This can be visualized as a relatively rigid body interacting with another rigid body, with the driving parameter for the binding interaction consisting of the release of bound water. Similar characteristics have been observed for a variety of nonantibody protein/protein and protein/peptide interactions. The results for NKG2D contrast remarkably with those for a set of antibody/protein interactions where induced fit has been demonstrated, as well as the TCR/MHCp interactions for which relatively large movement of the CDR loops of the TCR have been documented (Kjer-Nielsen et al., 2003; Reiser et al., 2003), leading to a view consistent with the thermodynamic measurements of other TCR (Wu et al., 2002).

Why have McFarland and Strong chosen to name the mechanism that prevails for NKG2D associations "rigid adaptation" rather than be satisfied with the designation "rigid lock-and-key?" The measurements of the NKG2D interaction with ULBP1 and with Rae-1β are consistent with a true rigid body lock-and-key mechanism. However, the results derived from NKG2D with MIC-A and MIC-B are suggestive of a small degree of conformational change on binding, not in the binding interface per se but rather involving stabilization of peripheral amino acid residues of the ligand, not the receptor. Consideration of this observation together with structural data indicating that MIC-A may order as many as 37 residues on binding to NKG2D leads the authors to take an intermediate position. How does NKG2D engage in such multifarious interactions without adjusting its structure? Part of the answer lies in the ability of two tyrosines in the core of the binding site to interact effectively yet distinctly with methionine, leucine, phenylalanine, and arginine residues on different ligands, as well as to form hydrogen bonds to disparate residues in the six different ligand sites examined. Thus, exploring the interaction of NKG2D with several ligands both structurally and thermodynamically has revealed the evolutionary adaptation of this NK receptor to productive association with multiple targets. Other receptors with a similar mechanism of degenerate recognition are sure to be found.

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