T-cell receptor binding kinetics in T-cell development and activation

Nicholas R.J. Gascoigne, Tomasz Zal and S. Munir Alam

T-cell activation is of central importance to the generation of an immune response and is also required as part of the host's ability to recognise self proteins. T cells are activated to differing extents by different ligands. Agonist ligands cause the full range of T-cell activation phenotypes – from activation of signalling cascades, to cytokine secretion or target cell killing, to T-cell proliferation. Partial agonists, which can differ from the agonist by as little as a single amino acid residue, can induce some of these responses but not all. Antagonist ligands can disable the signalling of an agonist ligand. These different types of interaction between ligand and T-cell receptor (TCR) also determine the developmental fate of maturing T cells. Much recent work has focused on how the T cell distinguishes between ligands. At least part of the answer lies in the kinetics of its binding to ligand.

The majority of T cells express a specific antigen receptor, the αβ T-cell receptor (TCR), which recognises a molecular complex present on the surface of cells. (The minority of T cells that express the γδ TCR and recognise a variety of ligands will not be dealt with here.) This complex comprises a peptide (p), either cytosolic or endocytosed, bound to a product of the major histocompatibility complex (MHCp). The peptide binds MHC in a specialised groove of the MHC protein and the MHCp complexes are presented to the TCR on the T-cell surface. Although activation of mature T cells by antigenic MHCp is highly specific, owing to the method of

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antigen presentation and other factors, the actual recognition event between TCR and MHCp shows substantial crossreactivity.

The activation or partial activation of T cells through the TCR is of great importance to the generation of an immune response, the survival of mature T cells and thymocyte development. It is an important part of the specific acquired immune system that eradicates cells infected with microorganisms. It also forms the basis of the host's ability to recognise self: T cells undergo positive and negative selection for the ability to recognise self MHCp complexes without becoming fully activated, thereby preventing an immune response to host tissues.

The TCR and MHC

TCR genes and proteins

The TCR has evolved to recognise MHC proteins and, under normal circumstances, T cells can only be activated following binding to MHCp complexes. The TCR consists of a clonally variable αβ heterodimer associated with the invariant chains of the CD3 complex: δε and γε heterodimers, and the ζζ homodimer. The CD3 components have immunoreceptor tyrosine-based activation motifs (ITAMs) that become phosphorylated after TCR ligation and recruit other signalling molecules to initiate a signalling cascade. The αβ component itself has no intrinsic signalling capacity. Each chain consists of a variable and a constant domain. The variable domain is encoded by a rearranged gene comprising components of the variable (V), diversity (D) and joining (J) minigenes (D is present in the β chain, but not the α chain). For reviews of TCR genes see Refs 1 and 2.

The TCR genes are rearranged during thymocyte development and are 'tested' for activity at different stages. The first stage, or 'β selection', tests the ability of β to associate with an invariant surrogate α chain called pre-Tα, which checks that the gene has been rearranged in-frame and that it can be expressed on the cell surface. The second checkpoint is 'positive selection', which occurs after β selection has signalled the cell to rearrange the α chain genes. At this stage, the αβ receptor is expressed on the cell surface and, in order for the T cell to be selected, its TCR must transduce a signal after recognition of thymic self MHCp complexes that fall within a certain affinity window. For reviews of thymocyte selection, see Refs 3 and 4.

MHC proteins

Two major types of MHC molecule are recognised by typical αβ TCRs: class I and class II. Class I molecules present cytosolic peptides, and thus are particularly suited for presenting antigens from intracellular viruses (Fig. 1). Class II molecules present peptides derived from endocytosed proteins, and thus tend to induce responses to extracellular proteins (Fig. 2) (reviewed in Ref. 5). Class I molecules comprise an α heavy chain (with α_1, α_2 and α_3 helices) and β_2 microglobulin (β_2m) light chain. The α chain encodes the two helices that form the sides and floor of the peptide-binding groove. Class II molecules consist of α (α_1 and α_2 helices) and β chains (β_1 and β_2 helices); the two chains are of similar size and each contributes one side of the binding groove. Class I is expressed on most cell types and is recognised by $CD8^+$ T cells. By contrast, class II is expressed on specialised antigen-presenting cells (APCs) and can be induced on other cells under certain conditions. Class II is recognised by $CD4^+$ T cells. Thus, $CD8^+$ T cells are said to be 'class I-restricted' and $CD4^+$ cells 'class II-restricted' on the basis of the class of MHC that their receptors recognise. CD4 interacts with class II at a site away from the TCR-interaction site, and CD8 interacts with a similar region of class I; the role of this interaction is to stabilise the TCR–MHCp complex.

MHC class I and class II both have a peptide-binding groove along the top (membrane-distal) face of the molecule. In class I molecules, the ends of the groove are closed so that the groove accommodates a peptide of 8–9 amino acids. Class II peptides can be much longer, since the ends of the groove are not closed, but the peptides tend to be 15–20 amino acids in length. Peptide amino acid side-chains can either interact with the MHC molecule or point out of the groove to be recognised by the TCR. Those interacting with the MHC often show 'anchor residues'; these are specific types of side-chains interacting with pockets in the floor of the binding groove. Thus, it is often possible to predict peptides that can bind to MHC molecules by searching for combinations of potential anchor residues that might fit the binding groove of a specific MHC molecule.

Structural basis of the TCR–MHCp interaction

Understanding the precise structural basis of the T-cell recognition of antigen has been much increased in the past few years, particularly

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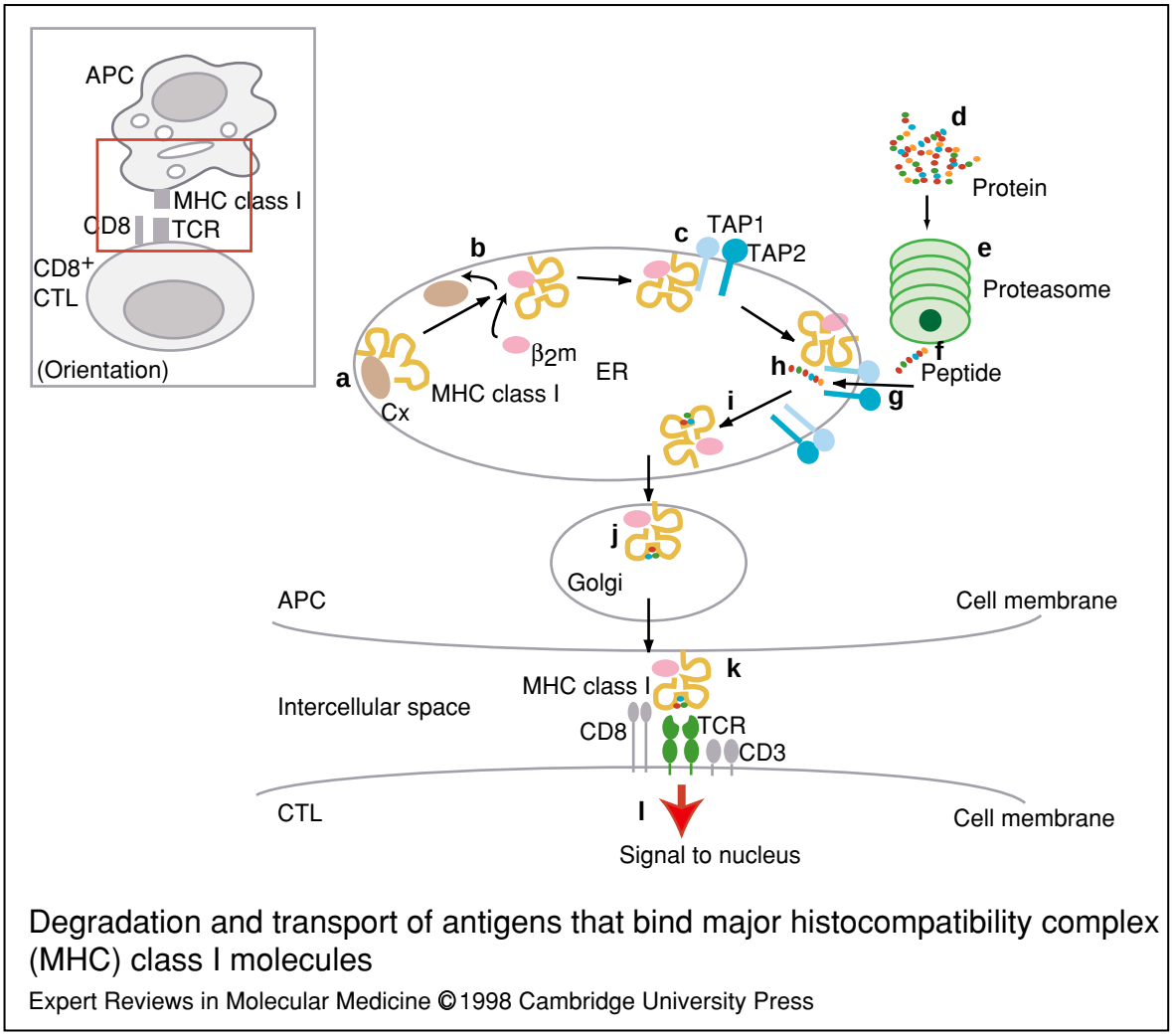

Degradation and transport of antigens that bind major histocompatibility complex (MHC) class I molecules

Figure 1. Degradation and transport of antigens that bind major histocompatibility complex (MHC) class I molecules. (a) In an antigen-presenting cell (APC), newly synthesised MHC class I molecules bind to calnexin (Cx), which retains them in a partially folded state in the endoplasmic reticulum (ER). (b) Binding of MHC class I molecules to β_2 microglobulin (β_2m) displaces Cx and allows binding of chaperonin proteins (calreticulin and tapasin; not shown). (c) The MHC class I–β_2m complex binds to the TAP complex (TAP1–TAP2), which awaits the delivery of peptides. (d) Peptides (e.g. from antigens) are formed from the degradation of cytosolic proteins ('self'-, pathogen- and tumour-derived proteins in the cytoplasm). (e) These are degraded by proteasomes into (f) short peptides. (g) Peptides are transported into the ER by the TAPs, where they meet the MHC class I–β_2m complex (h). This peptide binding in the antigenic groove of the MHC stablises the structure of the MHC class I molecule and (i) releases the TAP complex. (j) The fully folded MHC class I molecule with its peptide is transported to the cell surface via the Golgi apparatus. (k) Recognition of the MHC class I–peptide complex by the T-cell receptor (TCR) of an antigen-specific ($CD8^+$, $CD3^+$) cytotoxic T lymphocyte (CTL) takes place and (l) a signal transduction event activates effector functions in the class I-restricted T cell; this requires costimulation to occur (not shown) **(fig003smc)**. First published in: Stephen Man (1998) Human cellular immune responses against human papillomaviruses in cervical neoplasia. Exp. Rev. Mol. Med. 3 July, http://www-ermm.cbcu.cam.ac.uk/smc/txt001smc.htm

following the solving of crystal structures of several TCR–MHCp complexes (including several TCR–MHC class I structures and two TCR–MHC class II structures; Refs 6, 7, 8). Overall, it is clear that the TCR sits approximately at a diagonal relative to the peptide-binding groove of the MHC molecule. In this orientation, the 'complementarity-determining regions' (CDRs) of

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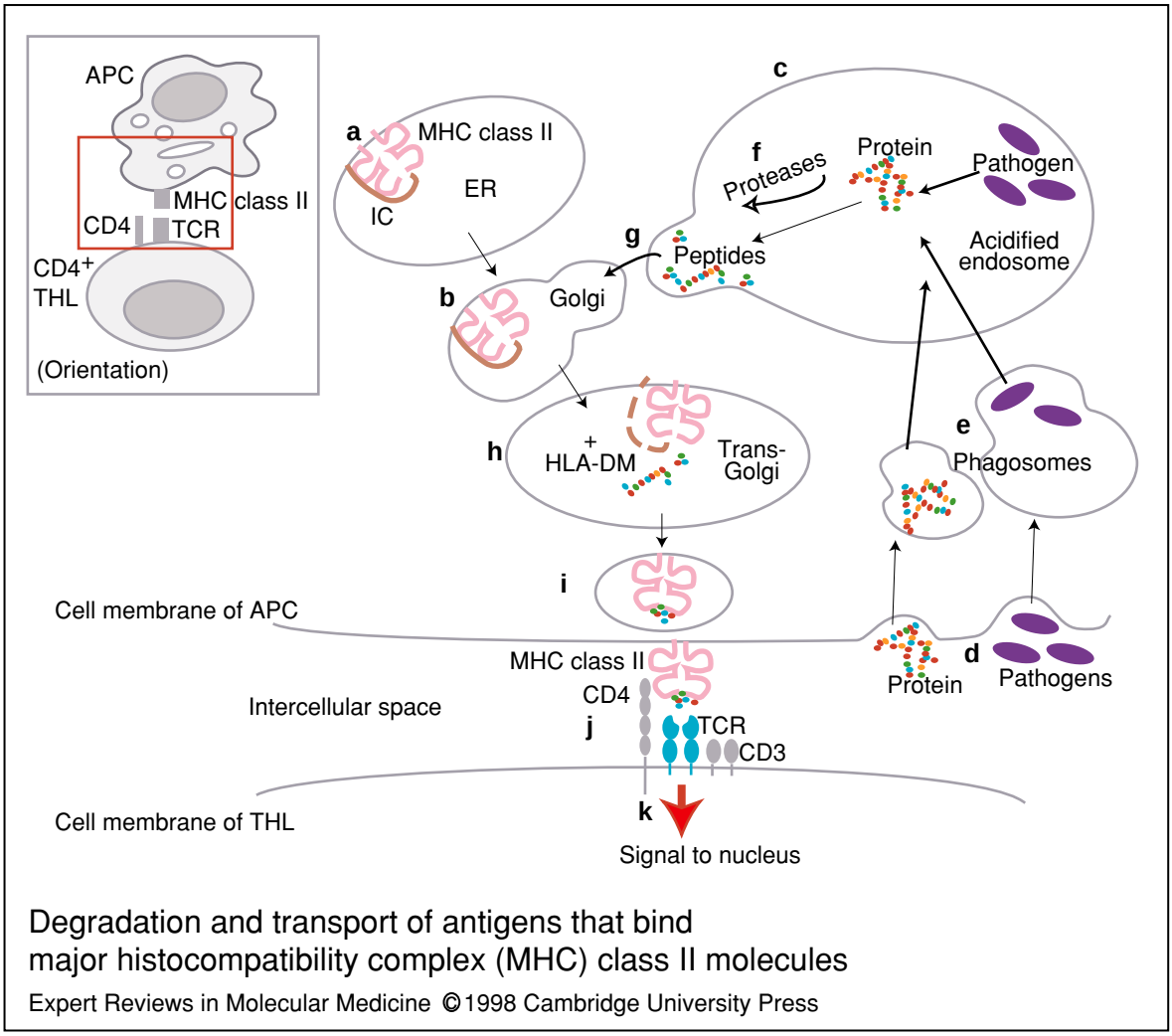

Degradation and transport of antigens that bind major histocompatibility complex (MHC) class II molecules

Figure 2. Degradation and transport of antigens that bind major histocompatibility complex (MHC) class II molecules. (a) In an antigen-presenting cell (APC), newly synthesised MHC class II molecules bind the invariant chain (IC), which prevents binding of peptides that are present in the endoplasmic reticulum (ER). (b) The IC allows transport of MHC class II molecules from the ER into the Golgi apparatus to acidified endosomes. (c) Endosomes contain peptides that are derived from either resident pathogens (e.g. bacteria) or (d) engulfed extracellular proteins (or pathogens) (e) in the phagosomes. (f) Proteases within the endosome degrade proteins into peptides. (g) The endosome fuses with the Golgi to form the trans-Golgi. (h) Here, the IC is cleaved and released from the MHC class II molecule. This allows the binding of peptides within the endosome to the peptide-binding cleft of the MHC molecules. A class II-like molecule (HLA-DM) binds to MHC class II molecules to facilitate the release of the IC (not shown). (i) The MHC class II–peptide complex is then transported to the cell surface of the APC for (j) recognition by the T-cell receptor (TCR) of ($CD4^+$, $CD3^+$) T helper (Th) cells and (k) intracellular signalling for activation. Recycling of MHC molecules and costimulation are not shown **(fig004smc)**. First published in: Stephen Man (1998) Human cellular immune responses against human papillomaviruses in cervical neoplasia. Exp. Rev. Mol. Med. 3 July, http://www-ermm.cbcu.cam.ac.uk/smc/txt001smc.htm

the TCR, which form the recognition domains, interact with specific regions of the α helices of the MHCp complex and with the peptide itself.

The CDR3 of the α and β chain of the TCR is formed during T-cell development from gene rearrangement between the V, D (in β) and J regions available in the germline. This region is therefore the most variable part of the TCR (Ref. 1). In the crystal structures, both of the TCR CDR3 sequences sit approximately over the central region of the peptide and make contact with peptide residues, with some contribution from

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regions of the MHC around this part of the peptide (Ref. 6). CDRs 1, 2 and 4 are germline-encoded and are thus much less variable than CDR3. These regions are most concerned with recognition of the MHC α helices (Ref. 9). CDRs 1 and 2 of the TCR α chain appear to have a major role in forming the interaction with MHC and in defining whether the TCR interacts mainly with MHC class I or class II molecules (Refs 9, 10). Vα accounts for the majority of the surface area between the TCR and MHC in most TCR–MHCp structures. Indeed, Vβ, in particular CDRs 1 and 2, sometimes has almost no contact with the MHC (Ref. 11). It seems that CDR1 and CDR2 of Vα form the most structurally conserved interactions with MHC, around which the rest of the TCR pivots (Ref. 9). When the TCR–MHCp structures are aligned using the MHC part of the structures, the TCR α chain CDRs are closely superimposed, whereas the β chain CDRs (especially CDRs 1, 2 and 4) are scattered (Ref. 6). CDR1α contacts residues on the α_1 and α_2 helices of class I (α_1 and β_1 of class II) and, in some cases, the N-terminus of the peptide. CDR2 interacts with residues on the MHC α_2/β_1 helix. Thus, although the specific TCR–MHC contacts vary from structure to structure, the pattern of binding between Vα and MHC is very conserved.

Activation of mature T cells by altered peptide ligands

Mature T cells can be stimulated not only by an antigen such as a peptide from a virus, but also by variants of this peptide, made either experimentally or by the virus itself. Such 'altered peptide ligands' (APLs) can cause a variety of different types of stimulation (Table 1) (Refs 3, 12). Strong agonists show a similar type of activation to the original antigen, and weak agonists are similar but require higher doses of ligand (or increased concentrations of co-receptor) to be equally effective. Partial agonists activate only some of the features of T-cell activation; for example, they can cause secretion of some

Table 1. Definition and effects of 'altered peptide ligands' (tab001ngs)

Ligand	Phenotype	Early signals
Agonist	Full activation of T cell; cytokine secretion; T-cell proliferation; CTL effector function	CD3ζ p23>p21[a]; recruitment and activation of ZAP-70
Weak agonist	Same as agonist, but requires more antigen for same degree of activation; slower activation kinetics	CD3ζ p23>p21; recruitment and activation of ZAP-70
Partial agonist	Induces some, but not all, effects of agonist (e.g. cytokine secretion but not proliferation)	Less CD3ζ p23 produced; deficient ZAP-70 activation
Antagonist	Inhibits activation by agonist; does not induce activation on its own	Only CD3ζ p21 produced
Survival ligand	Promotes long-term survival of naive T cells; natural ligands have not been defined yet, but MHCp is required for survival	Unknown, but naive peripheral T cells have CD3ζ p21
Null	No detectable activation	As for survival ligand but, in stimulated T-cell lines, no phospho-CD3ζ produced

[a] A low ratio of the p21 form of CD3ζ compared with the p23 form (i.e. p23>p21) is characteristic of full T-cell activation.
Abbreviations: CTL, cytotoxic T lymphocyte; MHCp, peptide bound to major histocompatibility complex; p21 and p23, phosphoprotein forms of the CD3ζ chain; ZAP-70, zeta-associated protein of 70 kDa (binds to phosphorylated CD3ζ chains).

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cytokines but not the whole range elicited by a strong agonist. No amount of partial agonist can bring the activation up to the level observed with a true agonist. For instance, a partial agonist can activate the T cell to produce interleukin 4 (IL-4) but, unlike the full agonist, will not activate the T cell to proliferate (Ref. 13). Antagonists are like partial agonists, in that they elicit some, but not all, aspects of T-cell activation. Their net effect is to interfere with ('antagonise') a response to an agonist ligand (Refs 3, 14).

Production of APL variants that antagonise the T-cell response is one method of immune evasion used by certain viruses (Refs 15, 16), but the major role of APLs in regulation of mature T cells under normal circumstances seems to be in the stimulation of survival signals. Low-level stimulation of naive T cells by MHCp ligands seems to be necessary for their survival (Ref. 17). Although natural ligands causing peripheral T-cell survival have not yet been identified, peptides that cause positive selection of thymocytes (see below) promote proliferation of naive cells in lymphopaenic mice (Ref. 18). The process therefore appears similar to thymocyte positive selection.

Studies involving in vitro stimulation of T cells with APLs have shown that these ligands activate the TCR to different degrees. Partial agonists and antagonists do not fully phosphorylate the CD3ζ chain, resulting in a higher ratio of the p21 form of ζ compared with the p23 form, where a low ratio is characteristic of full activation. Predominance of the p21 form results in lack of activation of a ζ-associated protein of 70 kDa (ZAP-70); ZAP-70 normally binds to phosphorylated CD3ζ chains and is involved in triggering the T-cell signalling cascade (Ref. 12). In at least one case, it has been shown that a weak agonist is able to stimulate T cells fully without detectable early signalling events. Over a longer period of time, it is able to stimulate late signalling events (C. Rosette et al., unpublished).

Activation of developing thymocytesby APLs

Developing thymocytes are subjected to two different forms of selection through their αβ TCRs. Negative selection occurs after recognition of a self MHCp ligand and results in activation-induced cell death (apoptosis). Cells with a TCR that recognises the MHC in the thymus, but not strongly enough to be negatively selected, undergo positive selection. This is a differentiation signal that upregulates the TCR and downregulates one of the co-receptors, CD4 or CD8. Thus, the cells differentiate from immature $CD4^+CD8^+$ to $CD4^+CD8^-$ or $CD4^-CD8^+$ mature thymocytes. Those cells that do not make a useful TCR because of nonfunctional rearrangements or because the expressed αβ TCR cannot be positively selected are doomed to die by neglect.

APLs have provided great insight into the role of TCR recognition in thymocyte development. Several groups have used peptides added to foetal thymic organ culture (FTOC) from either β_2m-deficient or peptide-transporter (TAP1)-deficient mice to study how peptide affects differentiation (Refs 3, 4). Cells from TAP1-deficient mice lack the ability to express class I MHCp unless peptide is added exogenously, because the normal route of MHC loading with peptide cannot occur in the absence of a peptide transporter (see Fig. 1). Similarly, in the absence of β_2m, MHC class I is not properly formed. Without exogenously added peptide (plus β_2m, for the β_2m-deficient cells), T-cell differentiation is blocked at the $CD4^+CD8^+$ stage. The addition of appropriate peptides ($\pm\beta_2$m) supports differentiation to the $CD4^+CD8^-$ or $CD4^-CD8^+$ mature stage.

When the thymus is obtained from a TCR-transgenic mouse, the addition of the antigenic peptide recognised by the TCR results in deletion of the $CD4^+CD8^+$ thymocytes. However, addition of certain APLs can result in positive selection. For example, in one TCR system (OT-1 TCR; recognising mouse MHC H-$2K^b$ bound to a peptide from ovalbumin), the addition of antagonist peptides results in positive selection (Refs 3, 19). In another system (P14 TCR; recognising mouse H-$2D^b$ bound to a peptide from lymphocytic choriomeningitis virus), partial or weak agonist peptides cause positive selection (Ref. 4). In the P14 TCR system, strong agonist ligands used at low concentrations can support positive selection (Refs 4, 20, 21); however, there is disagreement on whether these cases showed true positive selection since cells selected by agonists were not always fully functional (Refs 22, 23). In other cases, the cells selected by agonist ligands were reported to be functional (Refs 4, 24, 25).

Similar experiments have not been successful in MHC class II-restricted systems because of the lack of a suitable in vitro experimental system,

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but in vivo experiments have consistently shown positive selection by agonists as well as antagonists (Ref. 26). In one case, an antagonist changed the selection of a class II-restricted TCR from $CD4^+$ to $CD8^+$ cells (Ref. 27), suggesting that stronger activation signals lead to the development of class II-restricted cells, and weaker signals to the development of class I-restricted cells (Refs 28, 29, 30).

Experiments using class I-restricted systems clearly show that positive selection is supported by some APLs. Naturally occurring positive-selecting peptides have recently been found but, interestingly, their sequences show little similarity to the original antigenic ligands (Refs 31, 32). The naturally occurring peptides are generally weaker positive selectors than the APLs that are based on the original antigen, and naturally occurring peptides do not act as agonists or antagonists in the standard assays (Refs 31, 32). Our own binding data indicate that the affinity of naturally occurring peptides for the TCR is also much lower than that of the APLs (S.M. Alam et al., unpublished). Although no similar data are available for MHC class II-restricted systems, it has at least become clear that the development of a wide TCR repertoire requires the presence of a wide variety of class II MHCp ligands in the thymus. By contrast, limiting selection to a single MHCp species stunts development of the repertoire (Ref. 33).

Kinetics of TCR–MHCp binding

Recent studies have compared the kinetics of TCR binding to MHC in the presence of agonist ligands and APLs (Refs 34, 35, 36, 37, 38, 39; C. Rosette et al., unpublished; note that this list is incomplete and more information can be found reviewed in Ref. 36). These studies were performed using surface plasmon resonance measured in a BIAcore™ instrument (BIAcore AB, Uppsala, Sweden) using soluble versions of TCR and MHCp proteins at 25°C (see below for why the temperature may be significant). In BIAcore™ experiments on protein–protein binding, one protein is immobilised on a 'sensor chip' and the other is pumped over the surface of the chip (see http://www.biacore.com/biomol/biomol.shtml). As the protein in solution binds to the immobilised protein, there is a change in refractive index at the surface of the sensor chip that is detected by the instrument using polarised light. This is related to the mass of protein close to the surface of the chip and thus allows measurement of binding kinetics in real-time.

In general, a good correlation has been demonstrated between the kinetics of binding, in particular the off-rate (the rate of dissociation of the TCR from the MHCp complex) and the strength of T-cell activation (Table 2). Figure 3 shows data from the OT-1 system (measured at 37°C, see below). Together with Table 2, these data show that strong agonist ligands (e.g. H-2K^b–OVA) have the longest half-life of the complexes, antagonists (e.g. K^b–E1, K^b–R4) have an intermediate rate, and non-stimulatory ligands (e.g. K^b–polySer) have a very short half-life. In summary, these studies indicate that peptides that stimulate T cells well when bound to MHC also result in a longer period of TCR binding to MHCp. In addition, the differences in affinity between agonist and antagonist ligands were surprisingly small (Ref. 34) and, in some systems, there was no correlation between the biological effect and affinity (Ref. 37). An antagonist ligand has been reported with a significantly higher affinity than the corresponding agonist for that TCR although, unfortunately, kinetic studies have not been reported in this case (Ref. 40). The correlation between half-life and biological effect was absolute when ligands were compared that differ from the agonist ligand only by single amino acid changes in TCR contact residues. Although there is clearly an important kinetic component to T-cell activation, this cannot be the whole story (Refs 37, 38), as the following exceptions suggest.

Peptides exhibiting anomolous kinetics

In the MHC class I-restricted OT-1 system (Ref. 34), the antagonist/positive-selecting peptide V-OVA (RGY**N**Y**EK**L; contact residues in bold) contains the main TCR-contact residues from the OVA peptide (SII**N**F**EK**L), with the rest of the sequence derived from a non-stimulatory VSV peptide (RGY**V**Y**QG**L). This is therefore significantly different from the strong agonist OVA. Different peptide backbones are known to change the conformation of the MHC, such that the backbone of the α helices of the peptide-binding groove can change position, as well as individual amino acid side-chains (Refs 41, 42, 43). This can alter the TCR interactions with these molecules. In addition, the arginine at peptide residue 1 (P1) affects the interaction with the TCR since the side-chain of P1 points out of the MHC binding groove (Ref. 43).

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Table 2. Kinetics of TCR agonists and antagonists (tab002ngs)

TCR	Ligand[a]	Phenotype	K_D (μM)[d]	$T_{1/2}$ (s)[f]	Refs
OT-1	K^b–OVA	Agonist	6.2	33	34, 38
(MHC class I-restricted)	K^b–A2	Agonist	4.2	34.7	38
	K^b–G4	Intermediate agonist	10	99	[g]
	K^b–E1	Weak agonist/antagonist	20.8	10.3	34, 38
	K^b–V-OVA	Antagonist	22.9	18.7	34, 38
	K^b–R4	Antagonist	48.9	5.4	34, 38
	K^b–K4	Null[b]	>362	<3.5	34
	K^b–polySer	Null	ND	<3.5	38
2C	K^b–SIYR	Super-agonist	54	9.2	72
(MHC class I-restricted)	K^b–dEV8	Agonist	83	3.7	72
	K^b–p2Ca	Weak agonist	149	ND	72
	L^d–QL9	Agonist (allo)[c]	3.9	27.7	35
	L^d–p2Ca	Agonist (allo)	3.3	25.7	35
A6	A2–EtG	Super-agonist	4.2	3	44
(MHC class I-restricted)	A2–Tax	Agonist	0.82	7.5	39
	A2–P6PrA	Agonist	2.5	10.3	44
	A2–V7R	Weak agonist	8.1	1.5	39
	A2–P6MeG	Weak agonist	26	ND	44
	A2–P6A	Weak antagonist	116[e]	ND	44
2B4	E^k–MCC	Agonist	90	12	36
(MHC class II-restricted)	E^k–PCC	Agonist	80	8	36
	E^k–102S	Weak agonist	240	2	36
	E^k–102N	Weak agonist	320	1.6	36
	E^k–99R	Antagonist	500	0.15	36
	E^k–102G	Antagonist	1500	0.14	36
	E^k–99Q	Weak antagonist	2100	ND	36
3L2	E^k–Hb	Agonist	12	10.8	37
(MHC class II-restricted)	E^k–T72	Weak agonist	9.9	5.1	37
	E^k–D73	Weak agonist/antagonist	6.4	7.5	37
	E^k–I72	Antagonist	10/20	2.3–3.4	37
	E^k–A72	Weak antagonist	ND	<2	37

[a] Human MHC proteins are encoded by the human leukocyte antigen (HLA) region, whereas murine MHC proteins are encoded by the H-2 region. HLA-A2 (abbreviated to A2 above), H-2K (K) and H-2L (L) are class I molecules, and H-2E (E) is a class II molecule. The lower case superscripts b, d and k refer to allelic versions of H-2K, H-2L and H-2E molecules. The letters and numbers following the MHC name refer to special peptides bound to the MHC protein (see references).
[b] A null peptide is one that does not activate the T cell by any measurable method.
[c] An allo-MHC antigen is one derived from another MHC allele. In the 2C TCR system, this TCR developed in an animal with the H-2^b haplotype, so that it was selected for recognition of H-$2K^b$ bound to peptide. However, it also recognises H-$2L^d$ bound to peptide, where the L^d molecule is from an H-2^d mouse. The L^d–peptide complex is therefore an allo-antigen.
[d] The equilibrium dissociation constant (K_D) was determined in most cases from BIAcore™ measurements.
[e] Calculated from sedimentation equilibrium data.
[f] Half-life of the TCR–MHCp complex, calculated from the dissociation rate obtained from BIAcore™ measurements (ND, for not detectable, indicates that the dissociation rate was too fast to be measured).
[g] C. Rosette et al., unpublished.
Abbreviations: MHC, major histocompatibility complex; OVA, ovalbumin; p, peptide; TCR, T-cell receptor.

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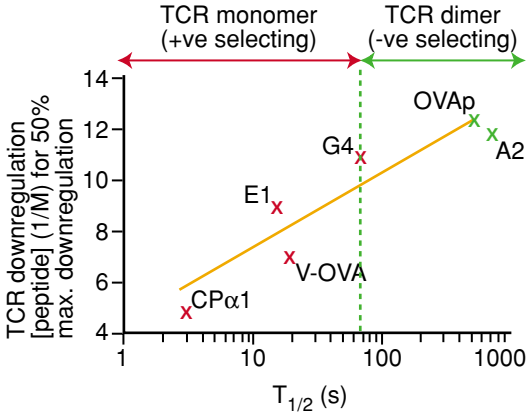
Another anomaly is observed with the G4 peptide. This is an agonist of intermediate potency, but does not show detectable early signals considered emblematic of agonist activation. Instead, signals appear to accumulate so that 'late events' are detectable, and T cells are activated by this ligand with delayed kinetics compared with OVA (C. Rosette et al., unpublished). The affinity measured for G4 is in line with its phenotype, but its off-rate is slower than for the strong agonist OVA. Measurements made at 37°C (Fig. 3) show that its half-life is not anomalous at that temperature.

In the A6 class I-restricted system, alterations in a peptide anchor residue of an antagonist class I MHCp complex that increased biological effectiveness (from antagonist, through weak agonist, to agonist) also increased the affinity and half-life of the complex. However, an alteration resulting in a super-agonist did not show higher affinity or longer half-life (Ref. 44).

In the MHC class II-restricted 3L2 system, an anomalous half-life is displayed by the weak agonist/antagonist D73 (Ref. 37). The natural residue at this position is glutamate 73 (p10), which is an anchor residue. Alterations at this position, including the change to aspartate in D73, have a major effect on T-cell activation (Ref. 13), suggesting that it affects the conformation of the MHC.

Finally, in the class I-restricted 2C system, the correlation between half-life and T-cell activation holds true when the MHC is constant, but not when different MHCs (even with same peptide) are compared (Refs 35, 46).

Although it is not clear why these ligands affect T-cell activation in a manner that is not consistent with their binding kinetics, at least some of the inconsistencies might disappear if measurements are made at physiological temperature (as with G4 in the OVA system). In other cases, differences in MHC structure caused by alteration of MHC-binding residues of the peptide might be important, as might TCR dimerisation or co-receptor recruitment (see below).



Correlation between the half-life of the TCR—MHCp complex and the degree of TCR downregulation

Expert Reviews in Molecular Medicine

Figure 3. Correlation between the half-life of the TCR–MHCp complex and the degree of TCR downregulation. Half-lives of binding of the OT-1 TCR to H-2K^b, plus the indicated peptides (CPα1, V-OVA, E1, G4, OVAp and A2), were measured at 37°C (Ref. 38; C. Rosette et al., unpublished; S.M. Alam et al., unpublished). TCR downregulation was measured by addition of various concentrations of the indicated peptide to a culture of OT-1 T cells with APCs. After culture, the level of the TCR was determined by fluorescence activated cell sorter and the concentration of peptide required for 50% of maximal downregulation (i.e. obtained with the agonist OVA peptide) was calculated. Positive (+ve) and negative (-ve) selection by different peptide ligands were determined using foetal thymic organ culture as described (Refs 19, 22, 31). Kinetics from BIAcore™ (BIAcore AB, Uppsala, Sweden) analysis fit well to a simple binding model, except for the strong agonist peptides OVAp and A2. These fit well to a dimerisation model of binding, which was therefore used to calculate the kinetics (Ref. 38). Abbreviations: MHCp, major histocompatibility complex bound to peptide; OVAp, ovalbumin peptide; TCR, T-cell receptor **(fig001ngs)**.

Dimerisation of the TCR

We have recently studied the effect of temperature on binding of the TCR to MHCp complexes, again using BIAcore™ analysis (see above). It had been noted in the OT-1 system that strong agonists bind TCRs with a very different kinetic profile at 37°C compared with the same ligands at 25°C. However, there was no difference in the kinetics of antagonist binding between 25°C and 37°C. At 25°C, agonists have a particular on- and off-rate, where the on-rate is generally slightly faster than for antagonist ligands, but without a very significant difference. By contrast, the off-rate for agonists is substantially slower than for

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antagonists (Refs 34, 38). However, at 37°C, both the on- and off-rate profiles changed for strong agonist ligands, and became substantially slower. However, the most striking observation was that the total mass of TCR protein that bound to MHCp was significantly increased at 37°C compared with 25°C. The increased binding at 37°C approached twofold higher than the amount bound at 25°C under otherwise identical conditions (Ref. 38). We have obtained very similar data with the P14 H-2D^b-restricted system (S.M. Alam et al., unpublished).

These binding data were analysed using various curve-fitting algorithms. At 37°C, the data fit poorly to a model for interaction between two reactants that form a simple one-to-one complex. By contrast, the equation was adequate to fit antagonist binding data under all conditions and it was effective at fitting agonist binding data at 25°C and below (Refs 34, 38). Other models that could be ruled out were those based on heterogeneic binding or simple conformational change: neither of these models could account for the increased mass of TCR protein that bound to the MHC–agonist peptide complex at 37°C. One model that could account for all the characteristics of the kinetics was dimerisation. The off-rates calculated with this model are greatly increased for strong agonists compared with antagonists and lead to a very good correlation between off-rate and biological activity in the OT-1 system (Fig. 3). This includes the intermediate agonist G4, which showed a very anomalous half-life at 25°C (discussed above) (C. Rosette et al., unpublished).

Two other groups have analysed kinetics of TCR binding to strong agonists at different temperatures using the BIAcore™ instrument. However, both of these labs found an increase in the rate of both the on- and off-phases of the interaction, and therefore an overall decreased affinity, at higher temperatures (Refs 45, 46). Furthermore, the reaction kinetics deviated from simple binding kinetics (Ref. 46). The interpretation for these data was that, at higher temperature, mobility in the CDRs allowed the TCR to reach the optimal conformation for MHC binding (i.e. 'induced fit') at a faster rate. At present, we are unable to explain fully the difference between these and our experiments, other than by differences between the TCR and MHC used in the experiments, or by differences in the degree of induced fit required for binding. In particular, the on- and off-rates for binding of the class I-restricted TCR used in one study (Ref. 45) are much faster at any given temperature than are either the OT-1 or P14 TCRs used in our studies (Ref. 38; S.M. Alam et al., unpublished).

Other evidence in support of TCR dimerisation comes from experiments using dimeric MHCp. Dimers are sufficient to activate T cells, although dimeric anti-TCR cannot activate, suggesting that formation of a TCR dimer in a particular manner is important for activation (Refs 47, 48). BIAcore™ data of dimeric MHCp binding to TCRs showed very similar kinetics to the 37°C OT-1 and P14 binding in that agonist MHCp apparently dimerised TCR at 37°C (Ref. 48). Analysis of the rate of TCR internalisation after ligand binding also supports dimerisation of the TCR (Ref. 49). Furthermore, staining of cytotoxic T lymphocyte (CTL) clones with class I MHCp tetramers [generated by binding biotinylated MHC class I molecules to streptavidin, which binds to four biotin molecules (Ref. 50)], revealed interesting temperature effects of binding to TCRs on the CTLs (Ref. 51). Some agonist or weak agonist tetramers bound more strongly at low temperatures than at high (i.e. similar to Refs 45, 46), whereas some strong agonists bound more strongly at 37°C (Ref. 51; i.e. similar to Ref. 38). The technique of quasi-elastic light scattering has also been used to demonstrate higher-order TCR structures (Ref. 52). These experiments showed that, at high concentrations, TCR and agonist MHCp complexes formed higher order structures, even up to hexameric complexes. Indeed, it should be noted that the BIAcore™ experiments using OT-1 and P14 would only have allowed detection of dimeric structures (Ref. 38). Higher order structures might, however, be formed on cell surfaces and there is evidence for lattices of TCR and co-receptor forming on cell surfaces during T-cell activation (Ref. 53).

The immunological synapse

In a dimerisation model, it can be envisaged that an initial binding of one TCR to MHCp is followed by the binding of a second TCR molecule, probably as a result of a conformational change induced after the initial binding. For many years, it has been assumed that the TCR complex expressed on the cell surface consists of a single αβ unit in association with the CD3 subunits (δε, γε, ζ_2). However, recent data from several groups indicate that there might in fact be two αβ units per complex (Refs 54, 55, 56). Thus, the

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dimerisation effect might be due to rearrangement of the two αβ units after MHCp binding, or it might be due to crosslinking of separate TCR–CD3 units (Ref. 38) (Fig. 4).

The 'immunological synapse' that forms during T-cell activation is a dynamic structure at the interface between a T cell and an APC. At this synapse, TCRs cluster in a central region on the T-cell surface (mirrored by MHCp clustering on the APC) and are surrounded by cell adhesion molecules that bind to their appropriate ligands on the APC (Refs 57, 58). Signalling molecules associated with TCRs and co-receptors are also clustered in the synapse. Dimerisation or multimerisation of the TCR is therefore likely to occur within the synapse region (Refs 38, 58) in a similar manner to that reported for CD4 lattice formation (Ref. 53).

Role of co-receptors

It is important to note that, in most of the studies described above, TCR and MHC molecules have been examined in membrane-free, soluble form. However, the αβ TCR exists as part of a complex containing the CD3 chains with associated signalling components. In addition, the co-receptors CD4 and CD8 play an important role in modulating or stabilising TCR–MHC interactions. Hence, a true affinity determination might require the TCR–CD3 complexes to be intact, and to include co-receptors and perhaps other signalling components.

Although the precise role of the co-receptors during TCR binding to MHCp is unclear, CD4 and CD8 molecules are believed to bind MHC molecules and enhance T-cell responses, and they may have a direct role in TCR–MHC complex formation (Refs 59, 60). In the mouse MHC class II system, it appears that CD4 is recruited to the TCR only in the case of agonist ligands but not for antagonist ligands, and that blocking of CD4 function results in turning an agonist into a partial agonist (Refs 61, 62). Data from our lab indicate a close apposition between TCR and CD4 only with agonist ligands (T. Zal et al., unpublished). However, other studies have found no effect on TCR–MHC stability when binding to multimeric ligands (Refs 63, 64, 65). This is in contrast to the effect of CD8 on binding of multimeric class I MHCp to TCR, where CD8 was absolutely required for binding of weak agonist ligands and clearly aided binding for strong agonist ligands (Ref. 66). Although soluble CD8 was found to stabilise TCR–MHCp binding (Ref. 67), this enhancement was not observed in a more-recent study, where distinct and independent weak binding interactions were observed between TCR–MHC and CD8–MHC complexes (Ref. 68). In one study, although monomeric MHCp was able to activate $CD8^+$ T cells, dimeric MHCp ligands were necessary for T-cell activation in the absence of CD8. This suggests that CD8 and the TCR bind the same MHC molecule and that linkage of the TCR and co-receptor in this way is sufficient for stimulation (Ref. 69).

A key issue that remains undecided is whether CD8 facilitates TCR–MHCp binding, or whether its effect is purely due to enhanced signal transduction, or both. CD8 molecules might interact only with complexes that are relatively long-lived (e.g. during TCR association with agonist ligands). They would thus be recruited later into pre-formed TCR–MHCp complexes, as proposed by Davis and colleagues (Refs 36, 62). Alternatively, CD8 molecules might have a role to play during TCR–MHCp formation, in which case, there will be a differential effect for each ligand in the presence of CD8. A recent report provides support for this view, since certain anti-CD8 antibodies were found to augment TCR–MHC interactions, with a greater effect on weak agonists than on strong agonists (Ref. 66).

The crystal structure of CD8αα bound to class I suggests that TCR and CD8 bind completely independently to class I, since the CD8αα stalk regions are predicted to lie almost perpendicular to the axis of the TCR–MHCp interaction (Ref. 70). However, modulation of TCR binding by CD8 remains possible, because the CD8 β chain has a shorter stalk than does the α chain, which could pull the CD8αβ complex close to the TCR–MHCp complex (Ref. 71). These stalks were not present in the proteins used for crystallisation.

A complex with CD8αβ might require readjustment of the TCR–MHC complex. Thus, one may predict that, under such restrained conditions, the overall tri-molecular (CD8–TCR–MHCp) complex will be relatively short-lived. In such a case, the true discrimination between agonist and antagonist ligands may occur at the tri-molecular complex stage.

Research in progress and outstanding research questions

Current research on the question of the role of kinetics in T-cell activation and thymocyte

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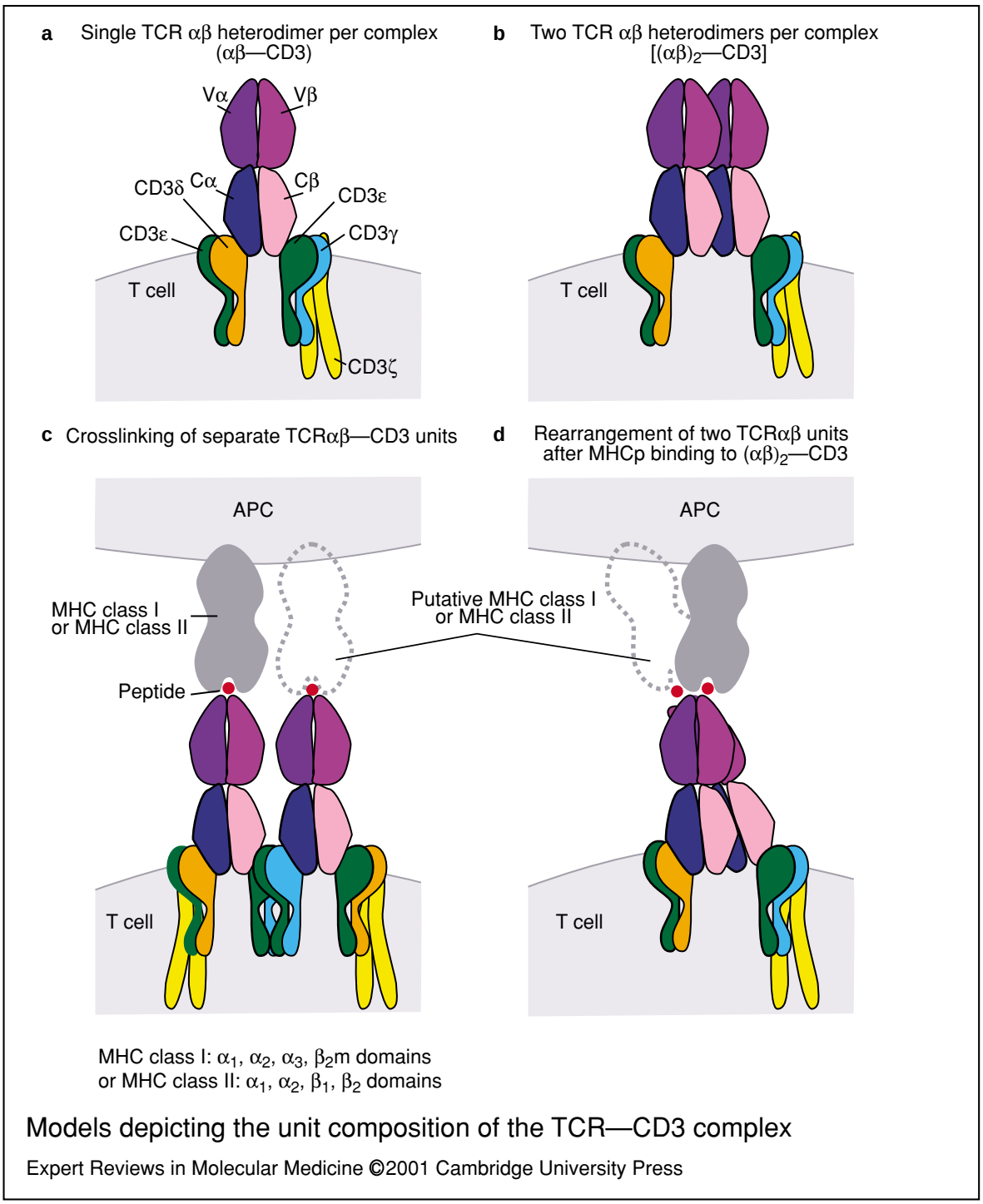

Models depicting the unit composition of the TCR—CD3 complex

Figure 4. Models depicting the unit composition of the TCR–CD3 complex. (a) The classical model with a single TCRαβ heterodimer per complex. (b) A dimerisation model showing two TCRαβ units per complex, as suggested by recent data (Refs 54, 55, 56). (c) The dimerisation effect that has been observed in BIAcore™ analysis of strong agonists at 37°C (Ref. 38) might be due to crosslinking of separate TCR–CD3 units. (d) Alternatively, it might be due to rearrangement of the two TCRαβ units after MHCp binding. The MHC depicted with a dotted line in (c) and (d) indicates that it is not clear whether its presence is necessary, a priori, for formation of the TCR dimer (see Ref. 38). Abbreviations: β_2m, β_2 microglobulin; APC, antigen-presenting cell; MHCp, major histocompatibility complex bound to peptide; TCR, T-cell receptor **(fig002ngs)**.

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development is moving away from the very reductionist approach of analysing the interactions of purified proteins in biochemical isolation. Our current work in the BIAcore™ system is concentrating on interactions of proteins on membranes. This work shows promise and should allow us to determine kinetics for interactions of whole TCR complexes. Another approach that we and others are pursuing is to use fusions of T-cell-surface proteins with a green fluorescent protein to analyse the movement and interactions of the proteins on the cell surface. Results from this work are starting to be published and it is safe to assume that they will revolutionise our understanding of T-cell activation.

Clinical implications

The relationship between the strength of signal upon T-cell recogniton of MHCp and the level and type of activation of the responding T cell has broad relevance in molecular medicine. This signal strength is a product of the strength of TCR binding described in this article, the quantity of the relevant peptide, the level of engagement of co-receptors and the degree of coupling of signalling machinery. As our understanding of the effects of the most variable of these – the peptide bound to the MHC molecule – on T-cell activation and development increases, so does the opportunity to take advantage of the differential effects of agonist and antagonist ligands in modulating T-cell activation. For example, it may become possible to induce tolerance to potential autoantigens by causing the responding T cells to be deleted in the thymus, or to divert such a response by appropriate antagonists. Such applications present an intriguing avenue for future studies in molecular medicine.

Concluding remarks

Several different avenues of investigation, including cellular immunology, in vitro BIAcore™ analysis and structural biology, have begun to converge to give an integrated picture of TCR interactions in T-cell activation. The kinetics of TCR–MHCp interactions play an important part in T-cell activation, particularly in distinguishing between almost identical ligands, but there are other levels of complexity that are just starting to become clear. The next few years will doubtless see a major leap in our understanding of the integration of signals leading to the numerous potential responses of the T cell.

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http://www.rcsb.org/pdb/

Gascoigne Lab website
http://www.scripps.edu/imm/gascoigne/

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Figure 1. Degradation and transport of antigens that bind major histocompatibility complex (MHC) class I molecules (fig003smc).
First published in: Stephen Man (1998) Human cellular immune responses against human papillomaviruses in cervical neoplasia. Exp. Rev. Mol. Med. 3 July, http://www-ermm.cbcu.cam.ac.uk/smc/txt001smc.htm
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First published in: Stephen Man (1998) Human cellular immune responses against human papillomaviruses in cervical neoplasia. Exp. Rev. Mol. Med. 3 July, http://www-ermm.cbcu.cam.ac.uk/smc/txt001smc.htm
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