

Table 9.1. Comparison of human T- and B-cells.

	T	B
% in peripheral blood	65-80	8-15
<b>ANTIGEN RECEPTORS:</b>		
Surface Ig	-	++
TCR/CD3	++	-
<b>OTHER RECEPTORS:</b>		
FcγRII	±	++
C3b receptors (CR1, CR2)	±	++
EBV receptors (=CR2)	-	++
Measles receptors	++	-
<b>OTHER MARKERS</b>		
MHC: Class I	++**	++
Class II	++**	++
<b>POLYCLONAL ACTIVATION</b>		
	anti-CD3	anti-Ig
	phytohemagglutinin (PHA)	<i>Staph. aureus</i> (str. Cowan 1)
	pokeweed mitogen (PWM)	pokeweed mitogen (PWM)
	concanavalin A	
	*superantigen (e.g. enterotoxin)	EBV

\*subpopulation; \*\*only activated cells; CD2=receptor forming sheep cell rosettes; CR1/2 = complement receptors 1/2; EBV = Epstein-Barr virus.

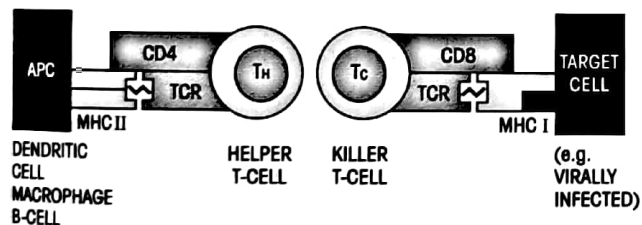


Figure 9.1. Helper and killer T-cell subsets are restricted by MHC class. CD4 on helpers contacts MHC class II; CD8 on killers associates with class I.

enterotoxins to act as polyclonal activators by stimulating all T-cells bearing certain T-cell receptor (TCR) Vβ families irrespective of their specificity for antigen.

### T-LYMPHOCYTES AND ANTIGEN-PRESENTING CELLS INTERACT THROUGH SEVERAL PAIRS OF ACCESSORY MOLECULES

The affinity of an individual TCR for its specific MHC-antigen peptide complex is relatively low

(figure 9.2) and a sufficiently stable association with the antigen-presenting cell (APC) can only be achieved by the interaction of complementary pairs of accessory molecules such as LFA-1/ICAM-1, CD2/LFA-3 and so on (figure 9.3). However, these molecular couplings are not necessarily concerned just with intercellular adhesion.

### THE ACTIVATION OF T-CELLS REQUIRES TWO SIGNALS

Antibodies to the TCR, either anti-idiotypic or anti-CD3, when insolubilized by coupling to Sepharose, will not fully activate resting T-cells on their own. Addition of interleukin-1 (IL-1) now readily induces RNA and protein synthesis, the cell enlarges to a blast-like appearance, interleukin-2 (IL-2) synthesis begins and the cell moves from G0 into the G1 phase of the mitotic cycle. Thus, two signals are required for the activation of a resting T-cell (figure 9.3). Antigen in association with MHC class II on the surface of APCs is clearly capable of fulfilling these requirements. Complex formation between the TCR, antigen and MHC provides signal 1 through the receptor-CD3 complex and this is greatly enhanced by the coupling of CD4 with the MHC. The T-cell is now exposed to a costimulatory signal 2 from the APC. Although this could be IL-1, it would appear that the most potent costimulators are likely to be B7 on the APC binding

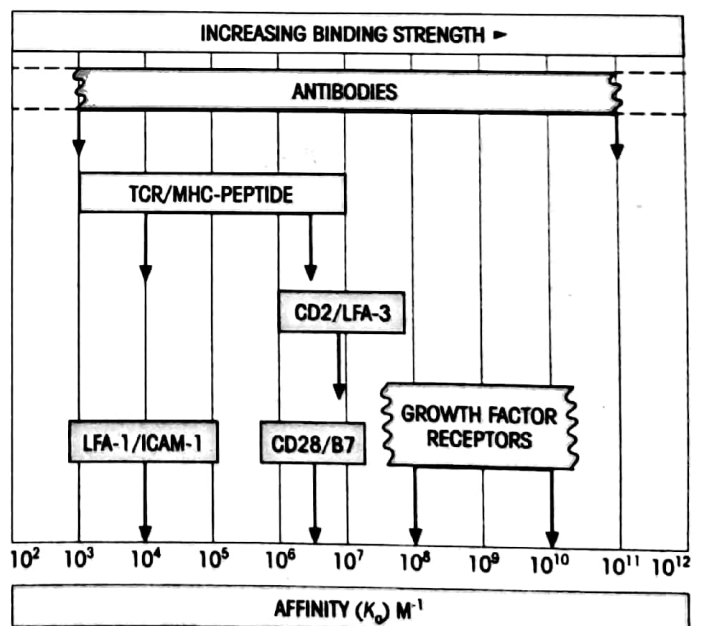
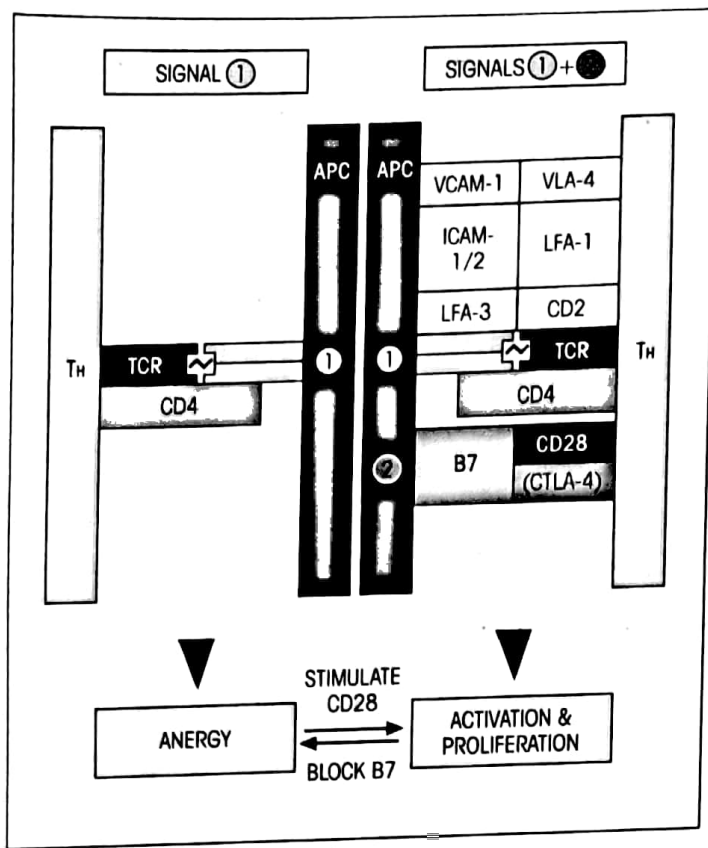


Figure 9.2. The relative affinities of molecular pairs involved in interactions between T-lymphocytes and cells presenting antigen. The range of affinities for growth factors and their receptors, and of antibodies, are shown for comparison. (Based on Davies M.M. & Chien Y.-H. (1993) *Current Opinion in Immunology* 5, 45.)



**Figure 9.3. Activation of resting T-cells.** Interaction of costimulatory molecules leads to activation of resting T-lymphocyte by antigen-presenting cell (APC) on engagement of the T-cell receptor (TCR) with its antigen-MHC complex. Engagement of the TCR signal 1 without accompanying costimulatory signal 2 leads to anergy. Note, a cytotoxic rather than a helper T-cell would, of course, involve coupling CD8 to MHC I. Engagement of CTLA-4 with B7 downregulates signal 1. LFA-1/2 = lymphocyte function associated molecule-1/2; ICAM-1/2 = intercellular adhesion molecule-1/2; VLA-4 = very late integrin antigen-4; VCAM-1 = vascular cell adhesion molecule-1. (Based on Liu Y. & Linsley P.S. (1992) *Current Opinion in Immunology* 4, 265-270.)

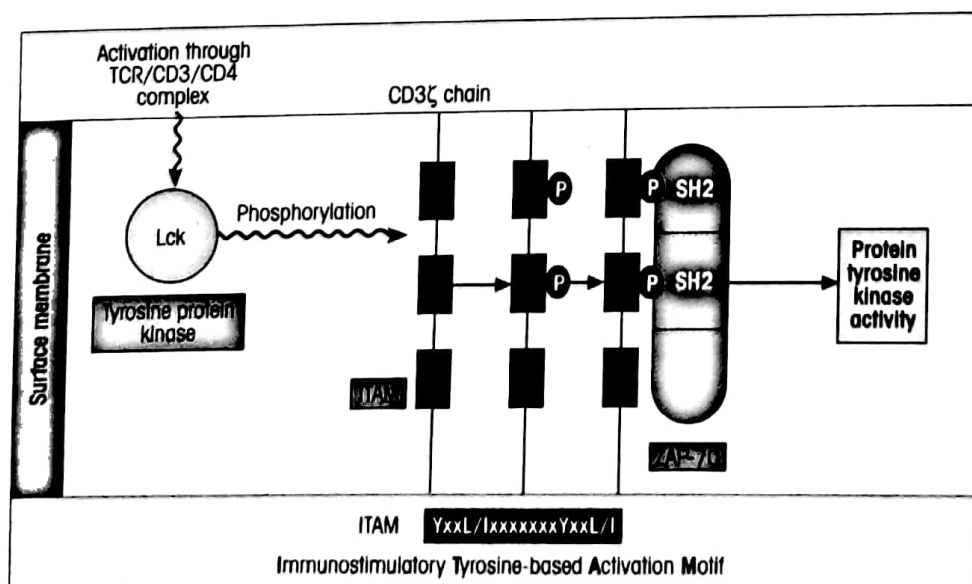
to CD28. Thus activation of resting T-cells can be blocked by anti-B7; surprisingly, this renders the T-cell **anergic**, i.e. unresponsive to any further stimulation by antigen. As we shall see in later chapters, the principle that two signals activate but one may induce anergy in an antigen-specific cell, provides a potential for targeted immunosuppressive therapy. Unlike resting T-lymphocytes, **activated T-cells proliferate in response to a single signal.**

Adhesion molecules such as ICAM-1, VCAM-1 and LFA-3 are not themselves costimulatory but augment the effect of other signals; an important distinction.

## PROTEIN TYROSINE PHOSPHORYLATION IS AN EARLY EVENT IN T-CELL SIGNALING

The conversion of phosphorylase *b* to the active *a* form by phosphorylation originally drew attention to the importance of protein phosphorylation for the regulation of cellular processes and we now know that transforming proteins of certain oncogenic retroviruses and receptors for a number of growth factors or hormones have intrinsic protein tyrosine kinase (PTK) activity. If such a kinase phosphorylates and thereby activates a kinase precursor, which in turn switches on a second kinase precursor and so on, one has the basis for an enzymic phosphorylation cascade which could amplify an initial signal just as the proteolytic enzyme cascade amplifies the triggering event of the complement system (cf. p. 11).

The initial signal for T-cell activation through the TCR is greatly enhanced by cross-linking the TCR with CD4 which brings the CD4-associated PTK, p56<sup>lck</sup>, close to the  $\zeta$ -chains of CD3. Immunostimulatory tyrosine-based activation motifs (ITAMs) on the  $\zeta$ -chains become phosphorylated and bind to the SH2



**Figure 9.4. Signals through the TCR/CD3/CD4/8 complex initiate a tyrosine protein kinase (TPK) cascade.** The TPK *lck* phosphorylates the tyrosine within the ITAM sequences of CD3  $\zeta$ -chains. These bind the  $\zeta$ -associated protein (ZAP-70) through its SH2 groups and this in turn acquires TPK activity for downstream phosphorylation of later components in the chain. The other CD3 chains each bear a single ITAM.

domains of ZAP-70 which now becomes an active PTK (figure 9.4) capable of initiating a series of downstream biochemical events.

The CD4 lck may also be responsible for activating the p21 ras protein. The key role of these tyrosine kinases is underlined by the ability of the PTK inhibitor herbimycin-A to block proximal TCR-mediated signaling events such as phosphatidylinositol turnover as well as later manifestations like IL-2 production.

## DOWNSTREAM EVENTS FOLLOWING TCR SIGNALING

### The phosphatidylinositol pathway

Within 15 seconds of TCR stimulation, **phospholipase C $\gamma$ 1**, an enzyme which activates the phosphatidylinositol pathway, is phosphorylated and its catalytic activity increased. This early increase in phospholipase C activity accelerates the hydrolysis of phosphatidylinositol diphosphate to diacylglycerol and inositol triphosphate (figure 9.5). The triphosphate binds to specific receptors on specialized calcium storage vesicles and triggers the release of Ca<sup>2+</sup> into the cytosol; this is supplemented by an influx from the external milieu. The raised Ca<sup>2+</sup> level has at least two consequences. First, it synergizes with diacylglycerol to activate **protein kinase C (PKC)**; second, it increases the activity of a very important enzyme, **calcineurin**.

### p21 ras function

Following TCR signaling, there is an early increase in the level of active p21 ras-GTP complexes which regulate pivotal enzymes, **mitogen-activated protein kinases (MAPK)** alias JNK or ERK, see legend figure 9.5) sequentially through Raf-1 and MAP kinase. Thus, the phosphorylation amplifying cascade would make Raf-1 a MAP kinase kinase or MAPKKK! MAP kinase may also be influenced by CD28 operating through phosphoinositide 3-kinase, and by protein kinase C and other factors as suggested in figure 9.5; it should be stressed, however, that these pathways are not yet inscribed in tablets of stone.

### Control of IL-2 gene transcription

Transcription of IL-2 is one of the key elements in preventing the signaled T-cell from lapsing into anergy and is controlled by multiple receptors for transcrip-

tional factors in the promoter region (figure 9.5). The key enzyme MAP kinase phosphorylates the Jun protooncogene, which then binds as a binary complex with Fos to the **AP-1** site, deletion of which abrogates 90% of IL-2 enhancer activity.

Under the influence of calcineurin, the cytoplasmic component of the nuclear factor of activated T-cells (**NFAT<sub>C</sub>**) becomes activated and translocates to the nucleus where it forms a binary complex with NFAT<sub>N</sub>, its partner which is constitutively expressed in the nucleus. The NFAT complex binds to two different IL-2 regulatory sites (figure 9.5). Note here that the calcineurin effect is blocked by the anti-T-cell drugs cyclosporin and FK506 (see chapter 17). PKC brings about the liberation of another transcriptional factor NF $\kappa$ B from its inhibitor I $\kappa$ B. In addition, the ubiquitous transcriptional factor **Oct-1** interacts with specific octamer binding sequence motifs.

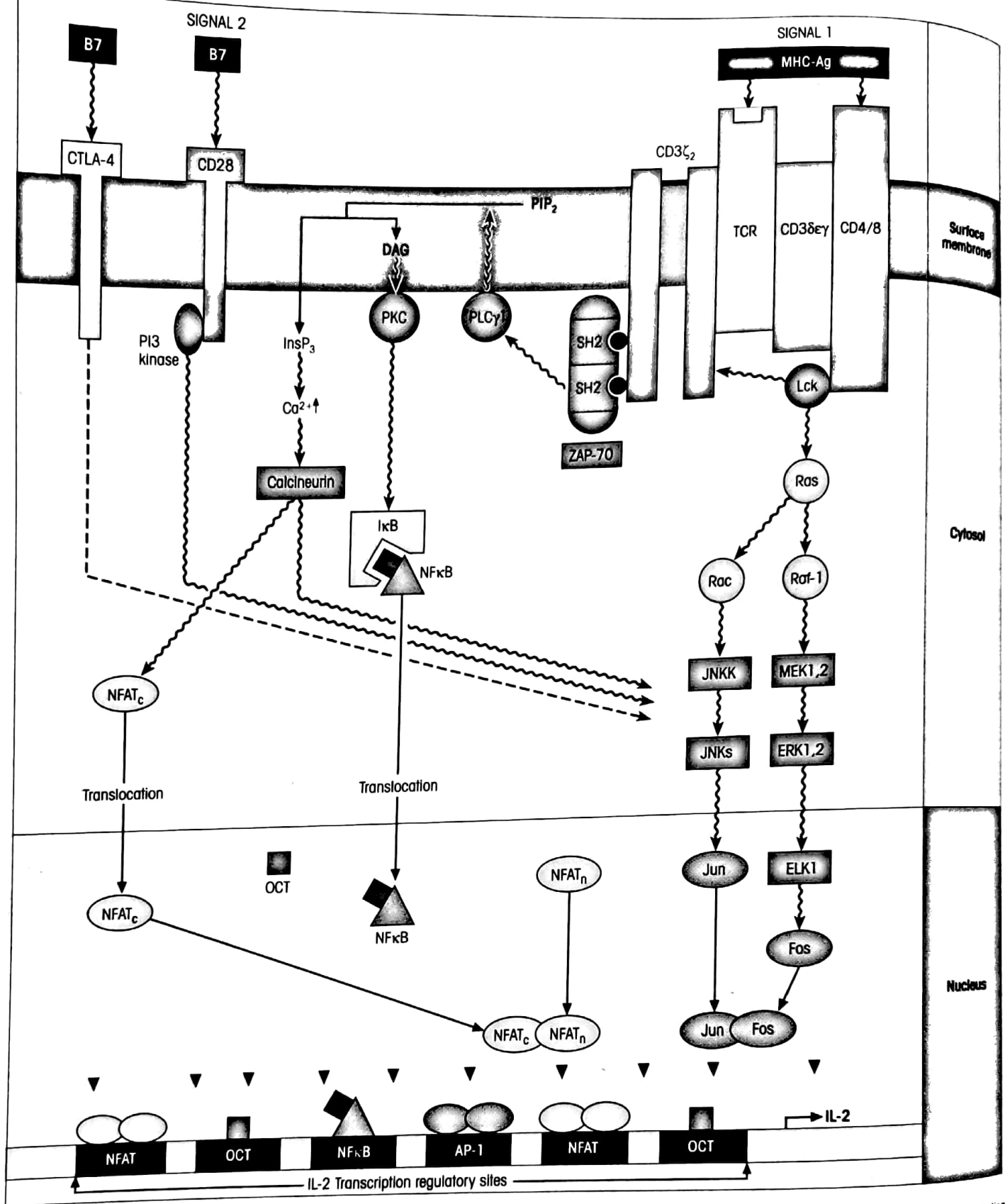
We have concentrated on IL-2 transcription as an early and central consequence of T-cell activation but finally, many genes become activated leading to T-cell proliferation and the synthesis of several other cytokines and their receptors (see chapter 10).

### Further thoughts on the control of T-cell triggering

#### A serial TCR engagement model for T-cell activation

I have already commented that the major docking forces which conjugate the APC and its T-lymphocyte counterpart must come from the complementary accessory molecules such as ICAM-1, LFA-1 and LFA-3/CD2, rather than through the relatively low affinity TCR-MHC/peptide links. Nonetheless, cognate antigen recognition by the TCR remains a *sine qua non* for T-cell activation. Fine, but how can as few as 100 MHC/peptide complexes on an APC, through their low affinity complexing with TCRs, effect the Herculean task of sustaining a raised intracellular calcium flux for the 60 minutes required for full cell activation? Any fall in calcium flux as may be occasioned by adding an antibody to the MHC, and NFAT<sub>C</sub> dutifully returns from the nucleus to its cytoplasmic location, so aborting the activation process.

Surprisingly, Valitutti and Lanzavecchia have shown that as few as 100 MHC/peptide complexes on an APC can downregulate 18 000 TCRs on its cognate T-lymphocyte partner. They suggest that each MHC/peptide complex can serially engage up to 200 TCRs. In their model, conjugation of an MHC/peptide dimer with two TCRs (cf. p. 99) activates signal transduction, phosphorylation of CD3  $\zeta$ -



**Figure 9.5. T-cell signaling leads to activation.** The signals through the MHC-antigen complex and costimulator B7, initiate a protein kinase cascade and a rise in intracellular calcium; these activate transcription factors which control entry in the cell cycle from G0 and regulate the expression of IL-2 and many other cytokines (see text). Lck = src-related tyrosine protein kinase (TPK); ZAP-70 =  $\zeta$  chain-associated protein kinase; PLC $\gamma$  = phospholipase C; PIP $_2$  = phosphatidylinositol diphosphate; InsP $_3$  = inositol triphosphate; DAG = diacylglycerol; PKC = protein kinase C; PI3 kinase = phosphoinositide-3 kinase; Raf-1 = a kinase which activates a series of kinases through MEK1,2 and ERK1,2 (extracellular signal regulated kinase) and ELK1, and finally the transcription factor Fos; Rac phosphorylates JNKK,

the kinase which activates JNK (Jun kinase) and finally the transcription factor Jun which, complexed with fos binds to the AP-1 transcription site; NFAT $_c$  is the resting cytoplasmic component of the nuclear factor of activated T-cells which translocates to the nucleus when activated by calcineurin and T-cells which translocates to the nucleus when activated by calcineurin and NFAT complexes with the nuclear component NFAT $_n$  and then binds to the NFAT transcription sites. PKC and possibly calcineurin release NF $\kappa$ B (nuclear factor  $\kappa$ B) from its inhibitor I $\kappa$ B whence it translocates to the nucleus and binds to a specific regulatory site; ● = phosphate group; ■ = octamer 1,2 transcription factors (an Oct binding factor OBF-1 acts as a B-cell co-activator and is essential for adequate antibody responses and germinal center formation as shown by 'knockouts'). B7 incites a negative signal through CTLA-4.

chain with subsequent downstream events, and then downregulation of those TCRs. Intermediate affinity binding favors dissociation of the MHC/peptide, freeing it to engage and trigger another TCR, so sustaining the required intracellular activation events. The model for **agonist** action would also explain why peptides giving interactions of lower or higher affinity than the optimum could behave as **antagonists** (figure 9.6). The important phenomenon of modified peptides behaving as **partial agonists** with differential effects on the outcome of T-cell activation, is addressed in the legend to figure 9.6.

#### *Damping T-cell enthusiasm*

I have frequently reiterated the premise that no self-respecting organism would permit the operation of an expanding enterprise such as a proliferating T-cell population without some sensible controlling mechanisms.

One such is the signal generated through B7-CTLA-4 coupling (figure 9.5), although the mecha-

nism is still unknown. Undoubtedly, phosphatases can undo the work of kinases and it is satisfying to note that the MAP kinase-mediated activation of gene transcription stimulates the production of a MAP kinase phosphatase with dual specificity for threonine and tyrosine phosphates which of course inactivates the MAP kinase itself.

Tempting though it might be, phosphatases should not automatically be equated with downregulation of a phosphorylation cascade. The observation that T-cell mutants lacking CD45 do not possess signal transduction capacity was at first sight deemed to be strange because CD45 has phosphatase activity and was thought thereby to downregulate signaling. However, the predominant form of the lck kinase in CD45-deficient cells is phosphorylated on tyrosine-505 which is a negative regulatory site for kinase activity; hence dephosphorylation by CD45 may activate the lck enzyme and the paradox is resolved.

#### **B-CELLS RESPOND TO THREE DIFFERENT TYPES OF ANTIGEN**