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Matthew Krummel, Christoph Wülfing, Cenk Sumen and Mark M. Davis

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SOCIETY
 Thirty-six views of T-cell recognition

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While much is known about the signalling pathways within lymphocytes that are triggered during activa-
tion, much less is known about how the various cell sur While much is known about the signalling pathways within lymphocytes that are triggered during activa-
tion, much less is known about how the various cell surface molecules on T cells initiate these events. To
address this While much is known about the signalling pathways within lymphocytes that are triggered during activation, much less is known about how the various cell surface molecules on T cells initiate these events. To address this, tion, much less is known about how the various cell surface molecules on T cells initiate these events. To address this, we have focused on the primary interaction that drives T-cell activation, namely the binding of a par address this, we have focused on the primary interaction that drives T-cell activation, namely the binding
of a particular T-cell receptor (TCR) to peptide–MHC ligands, and find a close correlation between
biological activ of a particular T-cell receptor (TCR) to peptide–MHC ligands, and find a close correlation between
biological activity and off-rate; that is, the most stimulatory TCR ligands have the slowest dissociation
rates. In general biological activity and off-rate; that is, the most stimulatory TCR ligands have the slowest dissociation rates. In general, TCRs from multiple histocompatibility complex (MHC) class-II-restricted T cells have half-lives rates. In general, TCRs from multiple histocompatibility complex (MHC) class-II-restricted T cells have
half-lives of 1–11s at 25 °C, a much narrower range than found with antibodies and suggesting a strong
selection for a half-lives of 1–11s at 25 °C, a much narrower range than found with antibodies and suggesting a strong
selection for an optimum dissociation rate. TCR ligands with even faster dissociation rates tend to be
antagonists. To selection for an optimum dissociation rate. TCR ligands with even faster dissociation rates tend to be antagonists. To observe the effects of these different ligands in their physiological setting, we made gene fusions of antagonists. To observe the effects of these different ligands in their physiological setting, we made gene fusions of various molecules with green fluorescent protein (GFP), transfected them into the relevant lymphocytes, lymphocytes, and observed their movements during T-cell recognition using multicolour video microbymphocytes, and observed their movements during T-cell recognition using multicolour video microscopy. We find that clustering of CD3 ζ –GFP and CD4–GFP on the T cell occurs concomitantly or slightly before the first ri scopy. We find that clustering of CD3 ζ –GFP and CD4–GFP on the T cell occurs concomitantly or slightly
before the first rise in calcium by the T cell, and that various GFP-labelled molecules on the B-cell side
cluster s before the first rise in calcium by the T cell, and that various GFP-labelled molecules on the B-cell side
cluster shortly thereafter (ICAM-1, class II MHC, CD48), apparently driven by T-cell molecules. Most of
this moveme cluster shortly thereafter (ICAM-1, class II MHC, CD48), apparently driven by T-cell molecules. Most of this movement towards the interface is mediated by signals through the co-stimulatory receptors, CD28 and LFA-1, and i this movement towards the interface is mediated by signals through the co-stimulatory receptors, CD28 and LFA-1, and involves myosin motors and the cortical actin cytoskeleton. Thus, we have proposed that the principal mec and LFA-1, and involves myosin motors and the cortical actin cytoskeleton. Thus, we have proposed that
the principal mechanism by which co-stimulation enhances T-cell responsiveness is by increasing the
local density of Tthe principal mechanism by which co-stimulation enhances T-cell responsiveness is by increasing the local density of T-cell activation molecules, their ligands and their attendant signalling apparatus. In collaboration wit local density of T-cell activation molecules, their ligands and their attendant signalling apparatus. In collaboration with Michael Dustin and colleagues, we have also found that the formation and stability of the TCR-pept collaboration with Michael Dustin and colleagues, we have also found that the formation and stability of the TCR-peptide–MHC cluster at the centre of the interaction cap between T and B cells is highly dependent on the dis the formation of a cell surface structure that is linked to and probably causal with respect to T-cell activation.

Keywords: T-cell receptor; Tcell; cell recognition; multiple histocompatibility complex; CD4;
Keywords: T-cell receptor; Tcell; cell recognition; multiple histocompatibility complex; CD4; cell recognition; multiple his
green fluorescent protein

1. INTRODUCTION

In 1830, the Japanese artist Hokusai began publishing his series of wood block prints entitled *Thirty-six views of Mt* In 1830, the Japanese artist Hokusai began publishing his
series of wood block prints entitled *Thirty-six views of Mt*
Fuji. While the concept seems tedious and obsessive at
first (and indeed it probably would have been series of wood block prints entitled *Thirty-six views of Mt Fuji*. While the concept seems tedious and obsessive at first (and indeed it probably would have been at the bands of say. Andy Warbol) the execution was not Fuji. While the concept seems tedious and obsessive at first (and indeed it probably would have been at the hands of say, Andy Warhol), the execution was not, and these images remain well known and even iconic to this first (and indeed it probably would have been at the hands of say, Andy Warhol), the execution was not, and these images remain well known and even iconic to this day (see www.csse.monash.edu.au/ \sim jwb/ukiyoe/hokusai. hands of say, Andy Warhol), the execution was not, and these images remain well known and even iconic to this
day (see www.csse.monash.edu.au/~jwb/ukiyoe/hokusai.
[html](http://www.csse.monash.edu.au/%7Ejwb/ukiyoe/hokusai.html)).Similarly, in modern biology it has often been
productive to examine complex natural phenomena from day (see www.csse.monash.edu.au/ \sim jwb/ukiyoe/hokusai. ar
html). Similarly, in modern biology it has often been ar
productive to examine complex natural phenomena from stu html). Similarly, in modern biology is
productive to examine complex natural
a variety of experimental perspectives.
One such phenomenon that we have productive to examine complex natural phenomena from
a variety of experimental perspectives.
One such phenomenon that we have focused on is that

a variety of experimental perspectives.

One such phenomenon that we have focused on is that

of transient cell–cell recognition, as exemplified by T

lymphocytes recognizing specific pentide–multiple histo-One such phenomenon that we have focused on is that
of transient cell-cell recognition, as exemplified by T
lymphocytes recognizing specific peptide-multiple histo-
compatibility complexes (MHC) on the surfaces of other of transient cell–cell recognition, as exemplified by T
lymphocytes recognizing specific peptide–multiple histo-
compatibility complexes (MHC) on the surfaces of other
cells. This process occurs continuously throughout the lymphocytes recognizing specific peptide–multiple histo-
compatibility complexes (MHC) on the surfaces of other
cells. This process occurs continuously throughout the life
of an individual and is vital for health. No evide compatibility complexes (MHC) on the surfaces of other
cells. This process occurs continuously throughout the life
of an individual and is vital for health. No evidence in
support of this statement is more sobering than th cells. This process occurs continuously throughout the life of an individual and is vital for health. No evidence in support of this statement is more sobering than the fact

that HIV infection, the subject of many of the papers in
this issue, specifically devastates CD4 T cells and thereby that HIV infection, the subject of many of the papers in
this issue, specifically devastates CD4 T cells and thereby
leaves the victim open to many diseases that would ordithat HIV infection, the subject of many of the papers in
this issue, specifically devastates CD4 T cells and thereby
leaves the victim open to many diseases that would ordi-
narily never be noticed. T cells as a distinct s this issue, specifically devastates CD4 T cells and thereby leaves the victim open to many diseases that would ordinarily never be noticed. T cells as a distinct subset of leaves the victim open to many diseases that would ordinarily never be noticed. T cells as a distinct subset of lymphocytes have been a central object of study in immunology since their discovery four decades ago narily never be noticed. T cells as a distinct subset of
lymphocytes have been a central object of study in
immunology since their discovery four decades ago
(Miller et al. 1961) and as a consequence all or most of lymphocytes have been a central object of study in
immunology since their discovery four decades ago
(Miller *et al.* 1961) and, as a consequence, all or most of
the key surface molecules that they rely on for recognition immunology since their discovery four decades ago (Miller *et al.* 1961) and, as a consequence, all or most of the key surface molecules that they rely on for recognition and auxiliary activities are known. Thus, T cells (Miller *et al.* 1961) and, as a consequence, all or most of the key surface molecules that they rely on for recognition and auxiliary activities are known. Thus, T cells represent an interesting (and clinically relevant) the key surface molecules that they rely on for recognition
and auxiliary activities are known. Thus, T cells represent
an interesting (and clinically relevant) model system for
studying how specific cell surface molecules and auxiliary activities are known. Thus, T cells represent
an interesting (and clinically relevant) model system for
studying how specific cell surface molecules mediate cell–
cell recognition an interesting (an
studying how spec
cell recognition.

2. RECEPTORS AND LIGANDS INVOLVED IN
2. RECEPTORS AND LIGANDS INVOLVED IN TORS AND LIGANDS INVOLV**I**
¤β T-CELL RECOGNITION

 $\alpha\beta$ T-CELL RECOGNITION
A surprisingly large number of cell surface molecules
near to play important roles in T-cell recognition **ap F-CELL RECOGNITION**
A surprisingly large number of cell surface molecules
appear to play important roles in T-cell recognition.
These include the α ⁸ form of T-cell receptors an anti-A surprisingly large number of cell surface molecules
appear to play important roles in T-cell recognition.
These include the $\alpha\beta$ form of T-cell receptors, an anti-
hody-like heterodimer that is responsible for recogni appear to play important roles in T-cell recognition.
These include the $\alpha\beta$ form of T-cell receptors, an anti-
body-like heterodimer that is responsible for recognizing
particular pentiele MHC complexes (on either clas These include the $\alpha\beta$ form of T-cell receptors, an anti-
body-like heterodimer that is responsible for recognizing
particular peptide–MHC complexes (on either class I or
class II MHC molecules) on another cell This is body-like heterodimer that is responsible for recognizing
particular peptide–MHC complexes (on either class I or
class II MHC molecules) on another cell. This is the key
event in the decision to recognize one cell versus a particular peptide-MHC complexes (on either class I or

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[{]Present address: Center for Immunology, UT Southwestern Medical $\begin{array}{l} \text{Author for correspondence (mdavis@cr} \text{Present address: Center for Immunol} \\\text{Center, Dallas, TX 75390-9093, USA.} \end{array}$

Figure 1. T-cell accumulation of MHC and ICAM and the formation of an immunological synapse. A representative primary
T cell is shown interacting with a supported lipid bilayer containing two types of GPI-linked molecules: Figure 1. T-cell accumulation of MHC and ICAM and the formation of an immunological synapse. A representative primary
T cell is shown interacting with a supported lipid bilayer containing two types of GPI-linked molecules T cell is shown interacting with a supported lipid bilayer containing two types of GPI-linked molecules: MHC (I-E^k) labelled with Oregon green dye and loaded with moth cytochrome c (88–103) peptide, and ICAM-1 labelle T cell is shown interacting with a supported lipid bilayer containing two types of GPI-linked molecules: MHC (I-E^k) labelled
with Oregon green dye and loaded with moth cytochrome c (88–103) peptide, and ICAM-1 labelle with Oregon green dye and loaded with moth cytochrome ϵ (88–103) peptide, and ICAM-1 labelled with Cy5 (red). The top
panel uses interference reflection microscopy (IRM) to delineate regions of close T-cell membrane—bi panel uses interference reflection microscopy (IRM) to delineate regions of close T-cell membrane—bilayer apposition, which
appear progressively darker as demonstrated by the outer ring in the T-cell contact at 1 min. The appear progressively darker as demonstrated by the outer ring in the T-cell contact at 1 min. The remaining panels show the
redistribution of the labelled proteins in the contact area by fluorescence video microscopy over redistribution of the labelled proteins in the contact area by fluorescence video microscopy over the course of 1 h. MHC is initially engaged in the tight outer edge of the contact, then actively accumulates into the centr MHC region, and serve as an adhesive fulcrum for T-cell attachment. From Grakoui *et al*. 1999.

 K_A (mM⁻¹)
Figure 2. Correlation of MHC cluster density in T-cell contacts with the solution kinetic parameters of the TCR-MHC-peptide
interaction. Pooling data from two different model antigen systems. Hb(64–76) (fill Figure 2. Correlation of MHC cluster density in T-cell contacts with the solution kinetic parameters of the TCR–MHC–peptide
interaction. Pooling data from two different model antigen systems, Hb(64–76) (filled squares) an Figure 2. Correlation of MHC cluster density in T-cell contacts with the solution kinetic parameters of the TCR–MHC–pep
interaction. Pooling data from two different model antigen systems, Hb(64–76) (filled squares) and mo interaction. Pooling data from two different model antigen systems, $Hb(64-76)$ (filled squares) and moth cytochrome ϵ (88–103) (open squares), demonstrates that when the kinetic factors of affinity (*a*), association to accumulated MHC. Thus, the half-life $(t_{1/2} = -\ln 2/k_{\text{off}})$ of the molecular interaction between TCRs and cognate MHC–
peptide ligands determines the final MHC density in T-cell contacts. From Grakoui *et al.* 1999.

the CD3 polypeptides, which are responsible for the These heterodimers are always expressed together with
the CD3 polypeptides, which are responsible for the
signal transduction (Irving & Weiss 1991), as the TCR
has no extends mic domain with that canability itself. As the CD3 polypeptides, which are responsible for the signal transduction (Irving & Weiss 1991), as the TCR has no cytoplasmic domain with that capability itself. As few as ten to 100 of the correct pentide-MHC complexes signal transduction (Irving & Weiss 1991), as the TCR
has no cytoplasmic domain with that capability itself. As
few as ten to 100 of the correct peptide–MHC complexes
are sufficient for recognition on the antigen-presentin has no cytoplasmic domain with that capability itself. As
few as ten to 100 of the correct peptide–MHC complexes
are sufficient for recognition on the antigen-presenting cell (APC). As with other membrane molecules that recognize ligands on other cells, the affinity of a TCR for cell (APC). As with other membrane molecules that
recognize ligands on other cells, the affinity of a TCR for
peptide–MHC is quite low, in the 10^{-4} to 10^{-6} m range
(table 1). The off-rates range between 0.25 and 0.0 recognize ligands on other cells, the affinity of a TCR for
peptide–MHC is quite low, in the 10^{-4} to 10^{-6} m range
(table 1). The off-rates range between 0.25 and 0.01s⁻¹
and in most systems an increasing off-rate (table 1). The off-rates range between 0.25 and $0.01s^{-1}$
and in most systems an increasing off-rate correlates with
loss of T-cell reactivity, even to the point where this can
result in interference with an otherwise and in most systems an increasing off-rate correlates with loss of T-cell reactivity, even to the point where this can result in interference with an otherwise stimulatory signal (antagonism). Association rates are generally quite low at result in interference with an otherwise stimulatory signal
(antagonism). Association rates are generally quite low at
 $500-10000 \text{ M}^{-1}\text{s}^{-1}$, and recent thermodynamic evidence
suggests that this may be due to a mechan (antagonism). Association rates are generally quite low at $500-10000 \, \text{M}^{-1}\text{s}^{-1}$, and recent thermodynamic evidence suggests that this may be due to a mechanism of binding in which somewhat disordered TCR-binding si $500-10000 \text{ M}^{-1}\text{s}^{-1}$, and recent thermodynamic evidence
suggests that this may be due to a mechanism of binding
in which somewhat disordered TCR-binding sites became *Phil. Trans. R. Soc. Lond.* B (2000)

These heterodimers are always expressed together with more ordered upon ligand binding (Willcox *et al.* 1999; the CD3 polypeptides, which are responsible for the Boniface *et al.* 1999). This may correspond to the 'induc more ordered upon ligand binding (Willcox *et al.* 1999;
Boniface *et al.* 1999) This may correspond to the 'induced more ordered upon ligand binding (Willcox *et al.* 1999;
Boniface *et al.* 1999). This may correspond to the 'induced
fit', mode, of binding, employed, by, DNA-recognition more ordered upon ligand binding (Willcox *et al.* 1999;
Boniface *et al.* 1999). This may correspond to the 'induced
fit' mode of binding employed by DNA-recognition
proteins which also have to discriminate between a larg Boniface *et al.* 1999). This may correspond to the 'induced fit' mode of binding employed by DNA-recognition proteins, which also have to discriminate between a large number of chemically similar molecules (Boniface *et* fit' mode of binding employed by DNA-recognition proteins, which also have to discriminate between a large number of chemically similar molecules (Boniface *et al.* 1999) 1999). number of chemically similar molecules (Boniface *et al.* 1999). Other molecules on the T-cell surface act to facilitate

1999).

Other molecules on the T-cell surface act to facilitate

recognition in ways that are not yet clear. These include

LEA-1 which recognizes ICAM-1 on other cells but appar-Other molecules on the T-cell surface act to facilitate
recognition in ways that are not yet clear. These include
LFA-1, which recognizes ICAM-1 on other cells but appar-
ently only after it has been converted into a 'high recognition in ways that are not yet clear. These include
LFA-1, which recognizes ICAM-1 on other cells but appar-
ently only after it has been converted into a 'high affinity
state' (still in the 1 um range) by TCR engage LFA-1, which recognizes ICAM-1 on other cells but apparently only after it has been converted into a 'high affinity state' (still in the $1 \mu m$ range) by TCR engagement. The role of the LFA-1-ICAM-1 interaction has long b ently only after it has been converted into a 'high affinity state' (still in the $1 \mu m$ range) by TCR engagement. The
role of the LFA-1-ICAM-1 interaction has long been
known to be important in T-cell adhesion, but it is also
increasingly clear that it has a signalling role as wel role of the LFA-1-ICAM-1 interaction has long l
known to be important in T-cell adhesion, but it is
increasingly clear that it has a signalling role as well.
CD4 and CD8 are molecules specific to the helpe own to be important in T-cell adhesion, but it is also
creasingly clear that it has a signalling role as well.
CD4 and CD8 are molecules specific to the helper or
totoxic subsets of T-cells, respectively, and bind class II

increasingly clear that it has a signalling role as well.
CD4 and CD8 are molecules specific to the helper or
cytotoxic subsets of T cells, respectively, and bind class II
or class I MHC^s. The affinity of CD4 for class I CD4 and CD8 are molecules specific to the helper or
cytotoxic subsets of T cells, respectively, and bind class II
or class I MHCs. The affinity of CD4 for class II MHC

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Figure 3. A model for checkpoints in T-cell activation. It is Figure 3. A model for checkpoints in T-cell activation. It is
suggested that the progression to effector functions involves
the ability of the TCR to (i) scan MHC complexes, then Figure 3. A model for checkpoints in T-cell activation. It is
suggested that the progression to effector functions involves
the ability of the TCR to (i) scan MHC complexes, then
(ii) form long-lived $TCR-**MHC**$ complexe suggested that the progression to effector functions involted ability of the TCR to (i) scan MHC complexes, then
(ii) form long-lived TCR-pMHC complexes thereby
recruiting CD4, which then (iii) initiates a cellular the ability of the TCR to (i) scan MHC complexes, then (ii) form long-lived TCR-pMHC complexes thereby recruiting CD4, which then (iii) initiates a cellular reorientation event stabilizing existing TCR complexes recruiting CD4, which then (iii) initiates a cellular
reorientation event stabilizing existing TCR complexes
while recruiting more TCRs to prolong the response. The
final configuration with TCRs highly localized into the reorientation event stabilizing existing TCR complexes
while recruiting more TCRs to prolong the response. The
final configuration with TCRs highly localized into the
middle of the interface is thus a result of traversing final configuration with TCRs highly localized into the middle of the interface is thus a result of traversing a number of checkpoints for TCR binding and pMHC recognition.

appears very weak and no direct binding measurements have been possible. For CD8, there are two conflicting appears very weak and no direct binding measurements
have been possible. For CD8, there are two conflicting
data sets. In any event, the presence of CD4 or CD8 has
the effect of amplifying the dose response of a given Tcel have been possible. For CD8, there are two conflicting
data sets. In any event, the presence of CD4 or CD8 has
the effect of amplifying the dose response of a given T cell
by ten to 100 times. Other key molecules on the Tdata sets. In any event, the presence of CD4 or CD8 has
the effect of amplifying the dose response of a given T cell
by ten to 100 times. Other key molecules on the T-cell
surface are CD28 and CTI A-4, both of which hind B the effect of amplifying the dose response of a given T cell
by ten to 100 times. Other key molecules on the T-cell
surface are CD28 and CTLA-4, both of which bind B7.1 by ten to 100 times. Other key molecules on the T-cell
surface are CD28 and CTLA-4, both of which bind B7.1
(CD80) and B7.2 (CD86) on other cells and serve to
respectively enhance or suppress T-cell activation surface are CD28 and CTLA-4, both of which bin
(CD80) and B7.2 (CD86) on other cells and se
respectively enhance or suppress T-cell activation.
CD28 ligation has a major 'co-stimulatory' eff CD30) and B7.2 (CD36) on other cells and serve to
spectively enhance or suppress T-cell activation.
CD28 ligation has a major `co-stimulatory' effect on
cell recognition, greatly amplifying activated T-cell

respectively enhance or suppress T-cell activation.
CD28 ligation has a major 'co-stimulatory' effect on
T-cell recognition, greatly amplifying activated T-cell

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responses and often being required for naive T-cell
responses A number of other T-cell molecules have also responses and often being required for naive T-cell
responses. A number of other T-cell molecules have also
been implicated in assisting recognition, about which responses. A number of other T-cell molecules have also been implicated in assisting recognition, about which much less is known. been implicated in assisting recognition, about which

3. DISSECTING THE CHOREOGRAPHY OF T-CELL 3. DISSECTING THE CHOREOGRAPHY OF T-CELL
RECOGNITION: USE OF ARTIFICIAL MEMBRANES

RECOGNITION: USE OF ARTIFICIAL MEMBRANES
How is it that these various molecules collaborate to
tow T-cell recognition? For many years it has been How is it that these various molecules collaborate to allow T-cell recognition? For many years it has been known that clustering TCRs on T cells or Igs on B cells allow T-cell recognition? For many years it has been
known that clustering TCRs on T cells or Igs on B cells
can artificially trigger activation. Subsequently, it has
been shown that CD3 ζ chimeras alone, when crossknown that clustering TCRs on T cells or Igs on B cells
can artificially trigger activation. Subsequently, it has
been shown that $CD3\zeta$ chimeras alone, when cross-
linked could induce T cells to become fully activated can artificially trigger activation. Subsequently, it has been shown that $CD3\zeta$ chimeras alone, when cross-
linked, could induce T cells to become fully activated.
This is analogous to other systems where receptor dimer been shown that $CD3\zeta$ chimeras alone, when cross-
linked, could induce T cells to become fully activated.
This is analogous to other systems where receptor dimer-
ization or multimerization initiates a signalling cascad linked, could induce T cells to become fully activated.
This is analogous to other systems where receptor dimer-
ization or multimerization initiates a signalling cascade
(EGF) The fact that this was not the whole story w This is analogous to other systems where receptor dimerization or multimerization initiates a signalling cascade (EGF). The fact that this was not the whole story was shown by the work of Monks *et al.* (1998) who used hi ization or multimerization initiates a signalling cascade (EGF). The fact that this was not the whole story was shown by the work of Monks *et al.* (1998), who used high resolution antibody staining of fixed T-B couples to (EGF). The fact that this was not the whole story was shown by the work of Monks *et al.* (1998), who used high resolution antibody staining of fixed T–B couples to show that TCR LFA-1 and CD28 were not randomly distrishown by the work of Monks *et al.* (1998), who used high resolution antibody staining of fixed T–B couples to show
that TCR, LFA-1 and CD28 were not randomly distri-
buted in the 'cans' at the interface but rather had a p resolution antibody staining of fixed T–B couples to show
that TCR, LFA-1 and CD28 were not randomly distri-
buted in the 'caps' at the interface but rather had a precise
'target' pattern, with TCR and CD28 occupying the ' that TCR, LFA-1 and CD28 were not randomly distributed in the 'caps' at the interface but rather had a precise
'target' pattern, with TCR and CD28 occupying the 'bull-
seve'. LFA-1 enriched in a ring around it on the T cel buted in the 'caps' at the interface but rather had a precise
'target' pattern, with TCR and CD28 occupying the 'bull-
seye', LFA-1 enriched in a ring around it on the T cell,
and a minor image of ligands on the B-cell sid 'target' pattern, with TCR and CD28 occupying the 'bull-
seye', LFA-1 enriched in a ring around it on the T cell,
and a minor image of ligands on the B-cell side (e.g.
MHC and B7 in the middle ICAM-1 outside) This seye', LFA-1 enriched in a ring around it on the T cell, and a minor image of ligands on the B-cell side (e.g. MHC and B7 in the middle, ICAM-1 outside). This and a minor image of ligands on the B-cell side (e.g. MHC and B7 in the middle, ICAM-1 outside). This striking pattern has been dubbed an 'immunological synance' and is associated with strong T_{cell} stimulation MHC and B7 in the middle, ICAM-1 outside). This
striking pattern has been dubbed an 'immunological
synapse' and is associated with strong T-cell stimulation.
In parallel with this work have been the efforts of iking pattern has been dubbed an 'immunological
napse' and is associated with strong T-cell stimulation.
In parallel with this work have been the efforts of
ichael Dustin and his colleagues to develop a dynamic

synapse' and is associated with strong T-cell stimulation.
In parallel with this work have been the efforts of
Michael Dustin and his colleagues to develop a dynamic In parallel with this work have been the efforts of
Michael Dustin and his colleagues to develop a dynamic
system to study the movements of membrane molecules
during T-cell recognition (Dustin *et al* 1998) Here Michael Dustin and his colleagues to develop a dynamic
system to study the movements of membrane molecules
during T-cell recognition (Dustin *et al.* 1998). Here,
surface molecules that have been engineered to have system to study the movements of membrane molecules
during T-cell recognition (Dustin *et al.* 1998). Here,
surface molecules that have been engineered to have
linid tails (so that they are laterally mobile) are labelled during T-cell recognition (Dustin *et al.* 1998). Here, surface molecules that have been engineered to have lipid tails (so that they are laterally mobile) are labelled with a particular fluorophore and then placed in a l surface molecules that have been engineered to have
lipid tails (so that they are laterally mobile) are labelled
with a particular fluorophore and then placed in a lipid
bilayer on a glass slide T cells are then denosited lipid tails (so that they are laterally mobile) are labelled
with a particular fluorophore and then placed in a lipid
bilayer on a glass slide. T cells are then deposited on this
slide and the movement of the different mol with a particular fluorophore and then placed in a lipid
bilayer on a glass slide. T cells are then deposited on this
slide and the movement of the different molecules is
observed by time-lapse video microscopy. The flat bilayer on a glass slide. T cells are then deposited on this
slide and the movement of the different molecules is
observed by time-lapse video microscopy. The flat
surface of the membrane on the slide ensures a flat interslide and the movement of the different molecules is
observed by time-lapse video microscopy. The flat
surface of the membrane on the slide ensures a flat inter-
face and bigh resolution. As shown in figure 1, when an observed by time-lapse video microscopy. The flat
surface of the membrane on the slide ensures a flat inter-
face and high resolution. As shown in figure 1, when an
MHC–pentide complex is labelled with fluorescein surface of the membrane on the slide ensures a flat inter-
face and high resolution. As shown in figure 1, when an
MHC-peptide complex is labelled with fluorescein face and high resolution. As shown in figure 1, when an MHC–peptide complex is labelled with fluorescein (green) and ICAM-1 is labelled with rhodamine (red), a
T cell recognizing these molecules quickly clusters them MHC–peptide complex is labelled with fluorescein
(green) and ICAM-1 is labelled with rhodamine (red), a
T cell recognizing these molecules quickly clusters them
underneath itself in a characteristic 'synanse' pattern (green) and ICAM-1 is labelled with rhodamine (red), a
T cell recognizing these molecules quickly clusters them
underneath itself in a characteristic 'synapse' pattern,
with the MHC in the interior and the ICAM-1 in a T cell recognizing these molecules quickly clusters them underneath itself in a characteristic 'synapse' pattern, with the MHC in the interior and the ICAM-1 in a underneath itself in a characteristic 'synapse' pattern,
with the MHC in the interior and the ICAM-1 in a
(largely) concentric ring around it. This exactly parallels
the findings of Monks *et al.* (1998) but has the added with the MHC in the interior and the ICAM-1 in a (largely) concentric ring around it. This exactly parallels the findings of Monks *et al.* (1998), but has the added advantage of showing the dynamics of the system as well (largely) concentric ring around it. This exactly parallels
the findings of Monks *et al.* (1998), but has the added
advantage of showing the dynamics of the system as well
as the fate of individual T cells. A particula the findings of Monks *et al.* (1998), but has the added advantage of showing the dynamics of the system as well as the fate of individual T cells. A particularly striking finding of this work (Grakoui *et al.* 1999) is t advantage of showing the dynamics of the system as well density of MHC clustering is proportional to the monofinding of this work (Grakoui *et al.* 1999) is that the density of MHC clustering is proportional to the monomeric half-life of the particular peptide–MHC complex with the TCR (as shown in figure $2c$)—thus establishing density of MHC clustering is proportional to the mono-
meric half-life of the particular peptide–MHC complex
with the TCR (as shown in figure $2c$)—thus establishing
a link between the kinetic measurements summarized in meric half-life of the particular peptide–MHC complex
with the TCR (as shown in figure $2c$)—thus establishing
a link between the kinetic measurements summarized in
table 1 with movements of populations of molecules on with the TCR (as shown in figure $2c$)—thus establishing
a link between the kinetic measurements summarized in
table 1 with movements of populations of molecules on cell surfaces. The data in figure 2 also firmly establish table 1 with movements of populations of molecules on
cell surfaces. The data in figure 2 also firmly establish
the importance of dissociation rate versus affinity or
association rate in determining the density of MHC cell surfaces. The data in figure 2 also firmly establish
the importance of dissociation rate versus affinity or
association rate in determining the density of MHC
clustering Thus the stability of TCR binding to pentide the importance of dissociation rate versus affinity or association rate in determining the density of MHC clustering. Thus the stability of TCR binding to peptide—MHC is critical to synapse formation and this in turn is li clustering. Thus the stability of TCR binding to peptide–
MHC is critical to synapse formation and this in turn is
linked to a robust T-cell response, presumably as an
increased density of ligand promotes a higher concen-MHC is critical to synapse formation and this in turn is
linked to a robust T-cell response, presumably as an
increased density of ligand promotes a higher concen-
tration of TCR–CD3 which in turn potentiates internal linked to a robust T-cell response, presumably as an increased density of ligand promotes a higher concentration of TCR–CD3, which in turn potentiates internal signalling signalling.

^a Range includes agonist peptides only for both MHC class I and II.

4. LABELLING T CELLS DIRECTLY: BEADS AND ELLING T CELLS DIRECTLY: BEADS A
GREEN FLUORESCENT PROTEINS **GREEN FLUORESCENT PROTEINS**
While extremely useful in defining and following the

While extremely useful in defining and following the
molecules on APCs that are the ligands for T-cell recogni-
tion, this type of system is not as well suited for studying While extremely useful in defining and following the
molecules on APCs that are the ligands for T-cell recogni-
tion, this type of system is not as well suited for studying
T-cell molecules. This is because it is the T-cel molecules on APCs that are the ligands for T-cell recognition, this type of system is not as well suited for studying T-cell molecules. This is because it is the T cell that is the active partner here with the movements of tion, this type of system is not as well suited for studying
T-cell molecules. This is because it is the T cell that is the
active partner here, with the movements of its molecules
driving the action of molecules on the AP T-cell molecules. This is because it is the T cell that is the active partner here, with the movements of its molecules driving the action of molecules on the APCs. The T-cell molecules are also linked to the cytoskeleton active partner here, with the movements of its molecules
driving the action of molecules on the APCs. The T-cell
molecules are also linked to the cytoskeleton in ways that
are not well defined and so it is not possible to driving the action of molecules on the APCs. The T-cell
molecules are also linked to the cytoskeleton in ways that
are not well defined and so it is not possible to reproduce
this on a glass slide. Therefore, we have label molecules are also linked to the cytoskeleton in ways that
are not well defined and so it is not possible to reproduce
this on a glass slide. Therefore, we have labelled intact T
cells in two different ways: (i) by attachi are not well defined and so it is not possible to reproduce
this on a glass slide. Therefore, we have labelled intact T
cells in two different ways: (i) by attaching large beads
 $(4-5 \text{ um})$ either via antibodies to cell s this on a glass slide. Therefore, we have labelled intact T
cells in two different ways: (i) by attaching large beads
 $(4-5 \mu m)$, either via antibodies to cell surface molecules
that are neutral with respect to activation cells in two different ways: (i) by attaching large beads

(4–5 μ m), either via antibodies to cell surface molecules

that are neutral with respect to activation or through

biotinylated lipids and streptavidin-coated b $(4-5 \mu m)$, either via antibodies to cell surface molecules
that are neutral with respect to activation or through
biotinylated lipids and streptavidin-coated beads
(Wülfing & Davis 1998) or (ii) using green fluorescent that are neutral with respect to activation or through
biotinylated lipids and streptavidin-coated beads
(Wülfing & Davis 1998), or (ii) using green fluorescent
protein (GEP) fusions to the cytoplasmic regions of (Wülfing & Davis 1998), or (ii) using green fluorescent protein (GFP) fusions to the cytoplasmic regions of (Wülfing & Davis 1998), or (ii) using green fluorescent
protein (GFP) fusions to the cytoplasmic regions of
various cell surface proteins (Wülfing *et al.* 1998;
Krummel *et al.* 2000) Using the first procedure we have protein (GFP) fusions to the cytoplasmic regions of various cell surface proteins (Wülfing *et al.* 1998; Krummel *et al.* 2000). Using the first procedure, we have been able to show that there is a distinct cell surface various cell surface proteins (Wülfing *et al.* 1998;
Krummel *et al.* 2000). Using the first procedure, we have
been able to show that there is a distinct cell surface
transport mechanism on T cells that is linked to the Krummel *et al.* 2000). Using the first procedure, we have been able to show that there is a distinct cell surface transport mechanism on T cells that is linked to the cytoskeleton and is triggered through the `co-stimulatory' transport mechanism on T cells that is linked to the
cytoskeleton and is triggered through the 'co-stimulatory'
receptors CD28 and LFA-1. This effect results in the
heads bound to the anterior of the T cell translocating t cytoskeleton and is triggered through the 'co-stimulatory'
receptors CD28 and LFA-1. This effect results in the
beads bound to the anterior of the T cell translocating to
the interface with an APC beginning at about 4 min receptors CD28 and LFA-1. This effect results in the
beads bound to the anterior of the T cell translocating to
the interface with an APC, beginning at about 4 min
after the first calcium flux and probably continuing beads bound to the anterior of the T cell translocating to
the interface with an APC, beginning at about 4 min
after the first calcium flux and probably continuing
throughout the first $30-60 \text{ min}$ of recognition We the interface with an APC, beginning at about 4 min
after the first calcium flux and probably continuing
throughout the first 30–60min of recognition. We believe
that this mechanism serves to deliver L-cell surface molafter the first calcium flux and probably continuing
throughout the first 30–60min of recognition. We believe
that this mechanism serves to deliver T-cell surface mol-
ecules and their subcellular components to the synapse throughout the first $30-60$ min of recognition. We believe
that this mechanism serves to deliver T-cell surface mol-
ecules and their subcellular components to the synapse
region and is likely to be the chief reason that that this mechanism serves to deliver T-cell surface molecules and their subcellular components to the synapse
region and is likely to be the chief reason that molecules
accumulate there much more quickly than one would
expect based on passive diffusion. It may also explain region and is likely to be the chief reason that molecules
accumulate there much more quickly than one would
expect based on passive diffusion. It may also explain
why cytoskeletal inhibitors such as cytocholasin D interaccumulate there much more quickly than one would
expect based on passive diffusion. It may also explain
why cytoskeletal inhibitors such as cytocholasin D inter-
fere so profoundly with T-cell recognition (and yet have expect based on passive diffusion. It may also explain
why cytoskeletal inhibitors such as cytocholasin D inter-
fere so profoundly with T-cell recognition (and yet have
no effect on APC: Wölfing et al. 1998). Similar obs why cytoskeletal inhibitors such as cytocholasin D inter-
fere so profoundly with T-cell recognition (and yet have
no effect on APC; Wülfing *et al.* 1998). Similar obser-
vations regarding a co-stimulation-driven transpor fere so profoundly with T-cell recognition (and yet have no effect on APC; Wülfing et al. 1998). Similar observations regarding a co-stimulation-driven transport mo effect on APC; Wülfing *et al.* 1998). Similar observations regarding a co-stimulation-driven transport mechanism have been reported by Viola *et al.* (1999), who have linked it to linid 'raft' movement using a choleravations regarding a co-stimulation-driven transport
mechanism have been reported by Viola *et al.* (1999), who
have linked it to lipid 'raft' movement using a cholera-
toxin-staining reagent. Thus, it may be that there is mechanism have been reported by Viola *et al.* (1999), who have linked it to lipid 'raft' movement using a choleratoxin-staining reagent. Thus, it may be that there is a cytoskeletal linkage to membrane rafts that transpor have linked it to lipid 'raft' movement using a choleratoxin-staining reagent. Thus, it may be that there is a cytoskeletal linkage to membrane rafts that transports

them (and their cargo) to the interface. In any event, the
transport of key recognition molecules to the T-cell–APC them (and their cargo) to the interface. In any event, the
transport of key recognition molecules to the T-cell–APC
interface would elevate the concentration of such molethem (and their cargo) to the interface. In any event, the transport of key recognition molecules to the T-cell–APC interface would elevate the concentration of such molecules and thereby potentiate T-cell activation. As n transport of key recognition molecules to the T-cell-APC
interface would elevate the concentration of such mole-
cules and thereby potentiate T-cell activation. As noted
elevatore (Cornall et al. 1998) even modest increas interface would elevate the concentration of such molecules and thereby potentiate T-cell activation. As noted elsewhere (Cornall *et al.* 1998), even modest increases in the number of signalling molecules can have a signi cules and thereby potentiate T-cell activation. As noted
elsewhere (Cornall *et al.* 1998), even modest increases in
the number of signalling molecules can have a significant
effect on the sensitivity of lymphocyte activat elsewhere (Cornall *et al.* 1998), even modest increases in the number of signalling molecules can have a significant effect on the sensitivity of lymphocyte activation. In light of this data, we have proposed that the entire enhanceeffect on the sensitivity of lymphocyte activation. In light
of this data, we have proposed that the entire enhance-
ment effect seen with co-stimulation in T cells may be due
to this type of effect (Willfing & Davis 1998 of this data, we have proposed that the entire
ment effect seen with co-stimulation in T cells is
to this type of effect (Wülfing & Davis 1998).
The second and more powerful approach to ent effect seen with co-stimulation in T cells may be due
this type of effect (Wülfing & Davis 1998).
The second and more powerful approach to the issue of
wy to follow the dynamics of molecules on T cells involves

to this type of effect (Wülfing & Davis 1998).
The second and more powerful approach to the issue of
how to follow the dynamics of molecules onTcells involves The second and more powerful approach to the issue of
how to follow the dynamics of molecules on Tcells involves
the use of GFP fusions. This method has proven widely
useful in a great number of biological applications how to follow the dynamics of molecules on Tcells involves
the use of GFP fusions. This method has proven widely
useful in a great number of biological applications
previously (Tsien 1998) but it can be argued that its mos the use of GFP fusions. This method has proven widely
useful in a great number of biological applications
previously (Tsien 1998), but it can be argued that its most
significant impact could be in the understanding of cell useful in a great number of biological applications
previously (Tsien 1998), but it can be argued that its most
significant impact could be in the understanding of cell
surface molecules The chemistry of soluble proteins c previously (Tsien 1998), but it can be argued that its most
significant impact could be in the understanding of cell
surface molecules. The chemistry of soluble proteins can be
readily studied in solution (at least in theo surface molecules. The chemistry of soluble proteins can be readily studied in solution (at least in theory), whereas the surface molecules. The chemistry of soluble proteins can be
readily studied in solution (at least in theory), whereas the
dynamics of cell surface proteins is not easily studied or can
only be studied to the sort of first readily studied in solution (at least in theory), whereas the
dynamics of cell surface proteins is not easily studied or can
only be studied to the sort of first approximation illustrated
by the data in table 1. These meas dynamics of cell surface proteins is not easily studied or can
only be studied to the sort of first approximation illustrated
by the data in table 1. These measurements are necessary in
order to understand the monomeric in only be studied to the sort of first approximation illustrated
by the data in table 1. These measurements are necessary in
order to understand the monomeric interaction dynamic by the data in table 1. These measurements are necessary in
order to understand the monomeric interaction dynamic
but are inherently unable to provide information regarding
the effects of movement in a bilayer-raft, the ef order to understand the monomeric interaction dynamic
but are inherently unable to provide information regarding
the effects of movement in a bilayer-raft, the effects of
polyvalency or the cooperativity between different but are inherently unable to provide information regarding
the effects of movement in a bilayer–raft, the effects of
polyvalency or the cooperativity between different types of
molecules (either directly or through their r the effects of movement in a bilayer-raft, the effects of
polyvalency or the cooperativity between different types of
molecules (either directly or through their respective
signalling pathways) or the consequences of spati polyvalency or the cooperativity between different types of
molecules (either directly or through their respective
signalling pathways), or the consequences of spatial segre-
gation at the interface (synapse formation). GE molecules (either directly or through their respective signalling pathways), or the consequences of spatial segregation at the interface (synapse formation). GFP fusions allow us to study individual molecules at 37° C gation at the interface (synapse formation). GFP fusions gation at the interface (synapse formation). GFP fusions
allow us to study individual molecules at 37° C in their
native context. Our early efforts in this regard showed that
ICAM-1-GFP on B cell APCs clustered rapidl allow us to study individual molecules at 37°C in their
native context. Our early efforts in this regard showed that
ICAM-1-GFP on B cell APCs clustered rapidly in the
synapse with a T cell beginning about 20s aft native context. Our early efforts in this regard showed that
ICAM-1-GFP on B cell APCs clustered rapidly in the
synapse with a T cell, beginning about 20s after the first
rise in intracellular free calcium (which we can mo ICAM-1-GFP on B cell APCs clustered rapidly in the synapse with a T cell, beginning about 20s after the first rise in intracellular free calcium (which we can monitor synapse with a T cell, beginning about 20s after the first
rise in intracellular free calcium (which we can monitor
simultaneously). This clustering of ICAM-1 did not occur
in cells that lacked co-stimulatory ligands and s rise in intracellular free calcium (which we can monitor
simultaneously). This clustering of ICAM-1 did not occur
in cells that lacked co-stimulatory ligands and served to
potentiate calcium signalling and by inference the simultaneously). This clustering of ICAM-1 did not occur
in cells that lacked co-stimulatory ligands and served to
potentiate calcium signalling and, by inference, the T-cell
response (Wülfing et al. 1998) in cells that lacked co-stimulatory ligands and served to potentiate calcium signalling and, by inference, the T-cell response (Wülfing *et al.* 1998).

Our most recent GFP work has focused on T-cell molresponse (Wülfing *et al.* 1998).
Our most recent GFP work has focused on T-cell molecules, where we have labelled CD3 ζ (the most important of the CD3 chains with respect to signalling) TCR α and Our most recent GFP work has focused on T-cell molecules, where we have labelled CD3 ζ (the most important of the CD3 chains with respect to signalling), TCR α and CD4. In none of these cases did the GEP fusion (to t ecules, where we have labelled CD3 ζ (the most important
of the CD3 chains with respect to signalling), TCR α and
CD4. In none of these cases did the GFP fusion (to the

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PHILOSOPHICAL
TRANSACTIONS $\overline{\overline{O}}$ cytoplasmic domain) have any noticeable effect on T-cell activation or signal transduction. To overcome the eytoplasmic domain) have any noticeable effect on T-cell
activation or signal transduction. To overcome the
problem of the non-planar nature of the T-cell–APC
couple, we have also constructed a video fluorescence activation or signal transduction. To overcome the
problem of the non-planar nature of the T-cell–APC
couple, we have also constructed a video fluorescence
microscopy apparatus which uses a moveable objective to problem of the non-planar nature of the T-cell-APC
couple, we have also constructed a video fluorescence
microscopy apparatus, which uses a moveable objective to
collect ontical sections $(20-30 \text{ at } 0.5-10 \text{ µm} \text{ thick})$ couple, we have also constructed a video fluorescence
microscopy apparatus, which uses a moveable objective to
collect optical sections $(20-30 \text{ at } 0.5-1.0 \mu\text{m} \text{ thick})$ through the cell couples, allowing us to view the interface collect optical sections $(20-30 \text{ at } 0.5-1.0 \mu\text{m}$ thick)
through the cell couples, allowing us to view the interface
in three dimensions (Krummel *et al.* 2000). Using this
system we have been able to show that TCR-CD through the cell couples, allowing us to view the interface
in three dimensions (Krummel *et al.* 2000). Using this
system, we have been able to show that TCR-CD3 clus-
ters rapidly to the synapse coincident with the firs in three dimensions (Krummel *et al.* 2000). Using this system, we have been able to show that TCR–CD3 clusters rapidly to the synapse, coincident with the first calcium flux and either remains there if activation is system, we have been able to show that TCR–CD3 clus-
ters rapidly to the synapse, coincident with the first
calcium flux, and either remains there if activation is
stable or dissinates and re-forms if it is not Furthermore ters rapidly to the synapse, coincident with the first calcium flux, and either remains there if activation is stable or dissipates and re-forms if it is not. Furthermore, CD4 GFP initially co-localizes to the centre of th stable or dissipates and re-forms if it is not. Furthermore, CD4 GFP initially co-localizes to the centre of the inter-
face but then is dispersed to the outer edges soon after-CD4 GFP initially co-localizes to the centre of the interwards. This runs contrary to the notion of CD4 as a coface but then is dispersed to the outer edges soon afterwards. This runs contrary to the notion of CD4 as a correceptor that serves to stabilize TCR-peptide–MHC with interactions (Janeway 1992) and instead suggests that it wards. This runs contrary to the notion of CD4 as a co-
receptor that serves to stabilize TCR–peptide–MHC
interactions (Janeway 1992) and instead suggests that it
may be acting to potentiate only the early phases of receptor that serves to stabilize TCR–peptide–MHC
interactions (Janeway 1992) and instead suggests that it
may be acting to potentiate only the early phases of
recognition CD4 GFP lacking its cytoplasmic domain interactions (Janeway 1992) and instead suggests that it
may be acting to potentiate only the early phases of
recognition. CD4 GFP lacking its cytoplasmic domain
also behaves in the same way so this pattern of movement may be acting to potentiate only the early phases of
recognition. CD4 GFP lacking its cytoplasmic domain
also behaves in the same way, so this pattern of movement
could not be orchestrated by a cytoskeletal linkage to recognition. CD4 GFP lacking its cytoplasmic domain
also behaves in the same way, so this pattern of movement
could not be orchestrated by a cytoskeletal linkage to
CD4 but instead might be the result of some type of also behaves in the same way, so this pattern of movement
could not be orchestrated by a cytoskeletal linkage to
CD4 but instead might be the result of some type of
physical exclusion occurring at the centre of the synapse could not be orchestrated by a cytoskeletal linkage to CD4 but instead might be the result of some type of physical exclusion occurring at the centre of the synapse (Krummel *et al* 2000) CD4 but instead might
physical exclusion occur:
(Krummel *et al.* 2000). **Krummel et al. 2000).**
5. CONCLUSION

A summary of what we have learned thus far is schematized in figure 3. Here we see a T-cell blast (that A summary of what we have learned thus far is
schematized in figure 3. Here we see a T-cell blast (that
is, one that had been in contact with ligand a week or so
beforehand) encountering an APC of the 'professional' schematized in figure 3. Here we see a T-cell blast (that
is, one that had been in contact with ligand a week or so
beforehand) encountering an APC of the `professional'
variety (B cells dendritic cells or macrophages) whi is, one that had been in contact with ligand a week or so
beforehand) encountering an APC of the 'professional'
variety (B cells, dendritic cells or macrophages), which
possesses co-stimulatory ligands. If an initial surve beforehand) encountering an APC of the 'professional' variety (B cells, dendritic cells or macrophages), which possesses co-stimulatory ligands. If an initial survey of the APC's membrane turns up a sufficient number ℓa variety (B cells, dendritic cells or macrophages), which possesses co-stimulatory ligands. If an initial survey of
the APC's membrane turns up a sufficient number (*ca*. ten
to 100) of peptide–MHC complexes that can stably bind
to its TCR_s small clusters of TCR–CD3–CD4 can for the APC's membrane turns up a sufficient number $(ea.$ ten
to 100) of peptide–MHC complexes that can stably bind
to its TCRs, small clusters of TCR–CD3–CD4 can form
perhaps nurely by chemical attraction, as seen in solutio to 100) of peptide–MHC complexes that can stably bind
to its TCRs, small clusters of TCR–CD3–CD4 can form
perhaps purely by chemical attraction, as seen in solution
by Reich et al. (1997): and this may allow the first rel to its TCRs, small clusters of TCR–CD3–CD4 can form
perhaps purely by chemical attraction, as seen in solution
by Reich *et al.* (1997); and this may allow the first release
of calcium from intracellular stores. It also le perhaps purely by chemical attraction, as seen in solution
by Reich *et al.* (1997); and this may allow the first release
of calcium from intracellular stores. It also leads to
synapse formation in which much larger cluste by Reich *et al.* (1997); and this may allow the first release
of calcium from intracellular stores. It also leads to
synapse formation, in which much larger clusters of TCR–
CD3 form, probably driven by the co-stimulatory synapse formation, in which much larger clusters of TCR–
CD3 form, probably driven by the co-stimulatory trans-
port mechanism described above, which also serves to
cluster MHC molecules on the APC side by an effect'. By CD3 form, probably driven by the co-stimulatory trans-
port mechanism described above, which also serves to
cluster MHC molecules on the APC side by an 'effect'. By
means and for reasons that are unknown synanse formaport mechanism described above, which also serves to cluster MHC molecules on the APC side by an 'effect'. By means and for reasons that are unknown, synapse formacluster MHC molecules on the APC side by an 'effect'. By
means and for reasons that are unknown, synapse forma-
tion is also accompanied by the segregation of LFA-1 and
other molecules to the outer ring area, where they ar means and for reasons that are unknown, synapse formation is also accompanied by the segregation of LFA-1 and other molecules to the outer ring area, where they are ionined by CD4. The establishment and maintenance of tion is also accompanied by the segregation of LFA-1 and
other molecules to the outer ring area, where they are
joined by CD4. The establishment and maintenance of
such a synapse for minutes or more correlates with a other molecules to the outer ring area, where they are
joined by CD4. The establishment and maintenance of
such a synapse for minutes or more correlates with a
robust (and irreversible) commitment to T-cell activation ioined by CD4. The establishment and maintenance of
such a synapse for minutes or more correlates with a
robust (and irreversible) commitment to T-cell activation
and the release of cytokines in the case of T belper cells such a synapse for minutes or more correlates with a robust (and irreversible) commitment to T-cell activation and the release of cytokines in the case of T helper cells (parallel studies on cytotoxic T cells have not yet been done, but will presumably yield similar results). arallel studies on cytotoxic T cells have not yet been
ne, but will presumably yield similar results).
Why is synapse formation important? We think that
cell recognition is an inherently 'noisy' process with a

done, but will presumably yield similar results).
Why is synapse formation important? We think that
T-cell recognition is an inherently 'noisy' process with a
large number of false starts. While this poisiness is prob-Why is synapse formation important? We think that
T-cell recognition is an inherently 'noisy' process with a
large number of false starts. While this noisiness is prob-
ably a consequence of the extreme sensitivity of T ce T-cell recognition is an inherently 'noisy' process with a
large number of false starts. While this noisiness is prob-
ably a consequence of the extreme sensitivity of T cells to
minute quantities of a particular antigen, large number of false starts. While this noisiness is probably a consequence of the extreme sensitivity of T cells to minute quantities of a particular antigen, this would make the danger of autoimmunity much more probable ably a consequence of the extreme sensitivity of T cells to minute quantities of a particular antigen, this would make the danger of autoimmunity much more probable. Thus, synapse formation may serve as a proofreading minute quantities of a particular antigen, this would make the danger of autoimmunity much more probable.
Thus, synapse formation may serve as a proofreading
process in which a larger area of the APC membrane is
surveyed for ligand, and only if additional 'examples' are Thus, synapse formation may serve as a proofreading
process in which a larger area of the APC membrane is
surveyed for ligand, and only if additional 'examples' are
found does the Tcell commit to the 'effector' phrase char process in which a larger area of the APC membrane is
surveyed for ligand, and only if additional 'examples' are
found does the T cell commit to the 'effector' phrase char-
acteristic of full activation surveyed for ligand, and only if additional 'examples' are
found does the Tcell commit to the 'effector' phrase char-
acteristic of full activation.

Clearly we are just at the beginning of a new apprecia-
on of how cell surface molecules on T cells scan cells in Clearly we are just at the beginning of a new apprecia-
tion of how cell surface molecules on T cells scan cells in
the body and arrive at a decision as to whether or not to Clearly we are just at the beginning of a new apprecia-
tion of how cell surface molecules on T cells scan cells in
the body and arrive at a decision as to whether or not to
achieve full activation. This decision depends o tion of how cell surface molecules on T cells scan cells in
the body and arrive at a decision as to whether or not to
achieve full activation. This decision depends on cytoske-
leton and signalling pathways inside the cell the body and arrive at a decision as to whether or not to
achieve full activation. This decision depends on cytoske-
leton and signalling pathways inside the cell as well as the
ligands available to the L-cell surface mole achieve full activation. This decision depends on cytoskeleton and signalling pathways inside the cell as well as the ligands available to the T-cell surface molecules. It is also dependent on synapse formation, although t leton and signalling pathways inside the cell as well as the
ligands available to the T-cell surface molecules. It is also
dependent on synapse formation, although the purpose of
this particular configuration of cell surfa ligands available to the T-cell surface molecules. It is also
dependent on synapse formation, although the purpose of
this particular configuration of cell surface molecules is
not clear, beyond the clustering of TCR–CD3 c dependent on synapse formation, although the purpose of
this particular configuration of cell surface molecules is
not clear, beyond the clustering of TCR–CD3 complexes
and the likely cascade of tyrosine kinase activity th this particular configuration of cell surface molecules is
not clear, beyond the clustering of TCR–CD3 complexes
and the likely cascade of tyrosine kinase activity that would ensue. Another mystery concerns the events and the likely cascade of tyrosine kinase activity that
would ensue. Another mystery concerns the events
leading up to the initial activation events—or rather,
what distinguishes these from the random 'noise' that a T would ensue. Another mystery concerns the events
leading up to the initial activation events—or rather,
what distinguishes these from the random `noise' that a T
cell encounters from the large number of nentide–MHCs leading up to the initial activation events—or rather,
what distinguishes these from the random 'noise' that a T
cell encounters from the large number of peptide–MHCs
on various cell surfaces that it would encounter every what distinguishes these from the random 'noise' that a T
cell encounters from the large number of peptide–MHCs
on various cell surfaces that it would encounter every
working day. It is also mysterious how co-stimulatory cell encounters from the large number of peptide–MHCs
on various cell surfaces that it would encounter every
working day. It is also mysterious how co-stimulatory
signals trigger the movement of cell surface molecules on various cell surfaces that it would encounter every
working day. It is also mysterious how co-stimulatory
signals trigger the movement of cell surface molecules
and what different signals through LFA-1 and CD28 are working day. It is also mysterious how co-stimulatory
signals trigger the movement of cell surface molecules
and what different signals through LFA-1 and CD28 are
doing (as they are synergistic they must be different in signals trigger the movement of cell surface molecules
and what different signals through LFA-1 and CD28 are
doing (as they are synergistic they must be different in
some way) Thus we have many phenomena operating in and what different signals through LFA-1 and CD28 are
doing (as they are synergistic they must be different in
some way). Thus we have many phenomena operating in
the same time-frame that are linked to the various cell doing (as they are synergistic they must be different in
some way). Thus we have many phenomena operating in
the same time-frame that are linked to the various cell
surface molecules and are somehow integrated to contrisome way). Thus we have many phenomena operating in
the same time-frame that are linked to the various cell
surface molecules and are somehow integrated to contri-
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