

Host factors influencing viral persistence

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 Host factors influencing viral persistence

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Dominik Wodarz² and Jan Pravsgaard Christensenⁱ

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With the aim of characterizing the antiviral immune response to a non-cytocidal virus, we studied the
outcome of lymphocytic choriomeningitis virus i With the aim of characterizing the antiviral immune response to a non-cytocidal virus, we studied the outcome of lymphocytic choriomeningitis virus infection in a number of gene knockout mouse strains.
Two virus strains di outcome of lymphocytic choriomeningitis virus infection in a number of gene knockout mouse strains.
Two virus strains differing markedly in their capacity to spread and replicate inside the murine host were used. Our results reveal that very different outcomes may be observed depending on virus strain and immunocompetence of the host. Thus while CD4⁺ cells are not critical during the initial phase of virus used. Our results reveal that very different outcomes may be observed depending on virus strain and
immunocompetence of the host. Thus while CD4⁺ cells are not critical during the initial phase of virus
control, infectio immunocompetence of the host. Thus while CD4⁺ cells are not critical during the initial phase of virus
control, infectious virus reappear in mice lacking CD4⁺ cells, B cells or CD40 ligand. Reappearance of
virus is ass control, infectious virus reappear in mice lacking CD4⁺ cells, B cells or CD40 ligand. Reappearance of virus is associated with impaired long-term CD8⁺ T-cell mediated immune surveillance, and the time to virus resurge virus is associated with impaired long-term CD8⁺ T-cell mediated immune surveillance, and the time to virus resurgence is inversely correlated to the replication rate of the virus. Our studies also reveal that interferon virus resurgence is inversely correlated to the replication rate of the virus. Our studies also reveal that
interferon- γ is a central cytokine, and depending on the rate of virus replication, mice lacking the ability
to interferon- γ is a central cytokine, and depending on the rate of virus replication, mice lacking the ability
to produce interferon- γ may develop either a severe, mostly fatal, T-cell mediated wasting syndrome or a
ch to produce interferon- γ may develop either a severe, mostly fatal, T-cell mediated wasting syndrome or a chronic infection characterized by long-term coexistence of antiviral cytotoxic T lymphocytes and infectious viru chronic infection characterized by long-term coexistence of antiviral cytotoxic T lymphocytes and infectious virus. Mathematical modelling indicates that these different outcomes may be explained in relatively simple mathe tious virus. Mathematical modelling indicates that these different outcomes may be explained in relatively
simple mathematical terms. This suggests that modelling may be used as a means to predict critical host
and virus p simple mathematical terms. This suggests that modelling may be used as a means to predict critical h
and virus parameters. Therefore, combining mathematical modelling with precise, quantitative, *in i*
analyses looks to be analyses looks to be a promising approach in addressing central quantitative issues in immunobiology.
Keywords: antiviral immunity; CD8⁺ Tcells; T-cell memory

1. INTRODUCTION

1. INTRODUCTION
The outcome of any infection is decided by a race
between the canacity of the infecting agent to invade and **EXECUTE 1. INTRODUCTION**
The outcome of any infection is decided by a race
between the capacity of the infecting agent to invade and
replicate inside the host and the ability of the host to The outcome of any infection is decided by a race
between the capacity of the infecting agent to invade and
replicate inside the host and the ability of the host to
respond efficiently applying a plethora of potential between the capacity of the infecting agent to invade and
replicate inside the host and the ability of the host to
respond efficiently, applying a plethora of potential
reflector systems. This statement is self-evident for replicate inside the host and the ability of the host to
respond efficiently, applying a plethora of potential
effector systems. This statement is self-evident for
infecting agents with the canacity to seriously disturb ho respond efficiently, applying a plethora of potential effector systems. This statement is self-evident for infecting agents with the capacity to seriously disturb host organ functions, but even in the case of, for example, effector systems. This statement is self-evident for infecting agents with the capacity to seriously disturb host organ functions, but even in the case of, for example, non-cytocidal viruses, this may be very important. Th infecting agents with the capacity to seriously disturb host if too many host cells in critical organ sites have become non-cytocidal viruses, this may be very important. Thus,
if too many host cells in critical organ sites have become
infected before an efficient immune attack is established,
the host may succumb from its own attemnt to cl if too many host cells in critical organ sites have become
infected before an efficient immune attack is established,
the host may succumb from its own attempt to clear the
infection (see for example Marker *et al.* 1976; infected before an efficient immune attack is established,
the host may succumb from its own attempt to clear the
infection (see, for example, Marker *et al.* 1976; Thomsen *et*
al. 1979: Leist *et al.* 1989: Fhl *et al.* the host may succumb from its own attempt to clear the infection (see, for example, Marker *et al.* 1976; Thomsen *et al.* 1979; Leist *et al.* 1989; Ehl *et al.* 1998). In this context, it infection (see, for example, Marker *et al.* 1976; Thomsen *et al.* 1979; Leist *et al.* 1989; Ehl *et al.* 1998). In this context, it should be remembered that non-cytocidal viruses may replicate to high titres without s al. 1979; Leist *et al.* 1989; Ehl *et al.* 1998). In this context, it should be remembered that non-cytocidal viruses may replicate to high titres without seriously affecting the host—thus allowing the stage to be set for should be remembered that non-cytocidal viruses may
replicate to high titres without seriously affecting the
host—thus allowing the stage to be set for the above
scenario. For the same reason, virus persistence may be replicate to high titres without seriously affecting the host—thus allowing the stage to be set for the above scenario. For the same reason, virus persistence may be established following infection with this category of scenario. For the same reason, virus persistence may be established following infection with this category of viruses. In this situation, the virus load becomes the result of a constant struggle between antiviral effector established following infection with this category of
viruses. In this situation, the virus load becomes the
result of a constant struggle between antiviral effector
systems and the canacity of the virus to avoid eliminati viruses. In this situation, the virus load becomes the result of a constant struggle between antiviral effector systems and the capacity of the virus to avoid elimination (Nowak & Bangham 1996). Hence analysing virus leve result of a constant struggle between antiviral effector
systems and the capacity of the virus to avoid elimination
(Nowak & Bangham 1996). Hence, analysing virus levels
under such conditions becomes a sensitive and biosystems and the capacity of the virus to avoid elimination
(Nowak & Bangham 1996). Hence, analysing virus levels
under such conditions becomes a sensitive and bio-
logically relevant parameter to evaluate the capacity of (Nowak & Bangham 1996). Hence, analysing virus levels
under such conditions becomes a sensitive and bio-
logically relevant parameter to evaluate the capacity of
the bost to maintain effective immune surveillance on a under such conditions becomes a sensitive and biologically relevant parameter to evaluate the capacity of
the host to maintain effective immune surveillance on a the host to maintain effective immune surveillance on a
*Author for correspondence (a.r.thomsen@immi.ku.dk).

long-term basis. However, a side-effect of this ongoing
hattle is chronic tissue damage. Therefore also under long-term basis. However, a side-effect of this ongoing
battle is chronic tissue damage. Therefore also under
these conditions immunopathology becomes the critical long-term basis. However, a side-effect of this ongoing
battle is chronic tissue damage. Therefore also under
these conditions, immunopathology becomes the critical
factor determining for how long the bost will survive battle is chronic tissue damage. Therefore also under these conditions, immunopathology becomes the critical factor determining for how long the host will survive these conditions, immunopathology becomes the critical
factor determining for how long the host will survive
without a breakdown of vital organ functions. Under
certain conditions the host may therefore be better off factor determining for how long the host will survive
without a breakdown of vital organ functions. Under
certain conditions the host may therefore be better off
aborting the immune response than maintaining a without a breakdown of vital organ functions. Under
certain conditions the host may therefore be better off
aborting the immune response than maintaining a
response that is insufficient for complete control. At the certain conditions the host may therefore be better off aborting the immune response than maintaining a response that is insufficient for complete control. At the present stage it suffices to conclude that although probresponse that is insufficient for complete control. At the response that is insufficient for complete control. At the present stage it suffices to conclude that although probably not the most common of events in nature, studying host interactions with non-cytocidal viruses may pro present stage it suffices to conclude that although probably not the most common of events in nature, studying
host interactions with non-cytocidal viruses may provide
valuable information about vital host defence mechanis ably not the most common of events in nature, studying
host interactions with non-cytocidal viruses may provide
valuable information about vital host defence mechanisms
and the factors involved in regulating their magnitud host interactions with non-cytocidal viruses may provide
valuable information about vital host defence mechanisms
and the factors involved in regulating their magnitude
and stability: information that may not be obtainable valuable information about vital host defence mechanisms
and the factors involved in regulating their magnitude
and stability; information that may not be obtainable
through analysis of more virulent infections. Hence and the factors involved in regulating their magnitude and stability; information that may not be obtainable through analysis of more virulent infections. Hence and stability; information that may not be obtainable
through analysis of more virulent infections. Hence
analysis of infections with non-cytocidal viruses has
served and continues to serve as a useful tool to address through analysis of more virulent infections. Hence
analysis of infections with non-cytocidal viruses has
served and continues to serve as a useful tool to address
central immunological issues such as memory anergy and analysis of infections with non-cytocidal viruses has
served and continues to serve as a useful tool to address
central immunological issues such as memory, anergy and
immune exhaustion (see for example Zinkernagel *et al* served and continues to serve as a useful tool to address
central immunological issues such as memory, anergy and
immune exhaustion (see, for example, Zinkernagel *et al.*
1993; Kundig *et al.* 1996)

2. THE VIRAL MODEL

Our group has for many years been using the murine 2. THE VIKAL MODEL
Qur group has for many years been using the murine
lymphocytic choriomeningitis virus (LCMV) infection as
a model to study various aspects of the interplay between Our group has for many years been using the murine
lymphocytic choriomeningitis virus (LCMV) infection as
a model to study various aspects of the interplay between
viruses and immune system (Volkert et al. 1975; Brolymphocytic choriomeningitis virus (LCMV) infection as
a model to study various aspects of the interplay between
viruses and immune system (Volkert *et al.* 1975; Bro-
Jorgensen 1978: Marker & Thomsen 1987: Thomsen & a model to study various aspects of the interplay between
viruses and immune system (Volkert *et al.* 1975; Bro-
Jorgensen 1978; Marker & Thomsen 1987; Thomsen &
Pfau 1993; Thomsen *et al.* 1998*a*). This virus is a natura Jorgensen 1978; Marker & Thomsen 1987; Thomsen &

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murine pathogen that, in the absence of a potent immune
response causes little or no damage to its primary host murine pathogen that, in the absence of a potent immune
response, causes little or no damage to its primary host.
This is evident from the fact that transplacentally and murine pathogen that, in the absence of a potent immune
response, causes little or no damage to its primary host.
This is evident from the fact that transplacentally and
neonatally infected mice may survive for the normal response, causes little or no damage to its primary host.
This is evident from the fact that transplacentally and
neonatally infected mice may survive for the normal life-
time of a mouse as persistent virus carriers, and early acquired infections probably constitute the most time of a mouse as persistent virus carriers, and such
early acquired infections probably constitute the most
common type of LCMV infection under natural condi-
tions. However a chronic virus carrier state may also be early acquired infections probably constitute the most
common type of LCMV infection under natural condi-
tions. However, a chronic virus carrier state may also be
produced by infection of adult T-cell-deficient mice common type of LCMV infection under natural conditions. However, a chronic virus carrier state may also be produced by infection of adult T-cell-deficient mice (Cole *et al* 1979: Christoffersen *et al* 1976: Moskophidis tions. However, a chronic virus carrier state may also be produced by infection of adult T-cell-deficient mice (Cole *et al.* 1972; Christoffersen *et al.* 1976; Moskophidis *et al.* 1987).

This observation is central for two reasons. First, it *dl.* 1987).
This observation is central for two reasons. First, it
demonstrates that the T-cell component of the immune
response is pivotal to the outcome of this infection both This observation is central for two reasons. First, it
demonstrates that the T-cell component of the immune
response is pivotal to the outcome of this infection, both
as regards immunonathology and virus control (Note: demonstrates that the T-cell component of the immune
response is pivotal to the outcome of this infection, both
as regards immunopathology and virus control. (Note:
we have deliberately chosen to use the word control rathe response is pivotal to the outcome of this infection, both
as regards immunopathology and virus control. (Note:
we have deliberately chosen to use the word control rather
than elimination based on the strong evidence sugge we have deliberately chosen to use the word control rather
than elimination based on the strong evidence suggesting we have deliberately chosen to use the word control rather
than elimination based on the strong evidence suggesting
that even in immunocompetent mice this infection may
never be completely cleared just reduced to a level b than elimination based on the strong evidence suggesting
that even in immunocompetent mice this infection may
never be completely cleared just reduced to a level below
the point of detection (Volkert & Lundstedt 1968: Ciur that even in immunocompetent mice this infection may
never be completely cleared just reduced to a level below
the point of detection (Volkert & Lundstedt 1968; Ciurea
et al. 1999)). Second as a consequence, this finding a never be completely cleared just reduced to a level below
the point of detection (Volkert & Lundstedt 1968; Ciurea
et al. 1999).) Second, as a consequence, this finding also
functions as a starting point for subsequent s the point of detection (Volkert & Lundstedt 1968; Ciurea *et al.* 1999).) Second, as a consequence, this finding also functions as a starting point for subsequent studies by showing that infection of adult mice with induce *et al.* 1999).) Second, as a consequence, this finding also functions as a starting point for subsequent studies by showing that infection of adult mice with induced defects in their immune response may be used to study c functions as a starting point for subsequent studies by
showing that infection of adult mice with induced defects
in their immune response may be used to study critical T-
cell effector functions, and their regulation. Thu showing that infection of adult mice with induced defects
in their immune response may be used to study critical T-
cell effector functions and their regulation. Thus, a
similar outcome to that obtained in completely T-cel in their immune response may be used to study critical T-
cell effector functions and their regulation. Thus, a
similar outcome to that obtained in completely T-cell-defi-
cient mice is to be found in mice deficient of CD8 cell effector functions and their regulation. Thus, a
similar outcome to that obtained in completely T-cell-defi-
cient mice is to be found in mice deficient of $CD8^+$ Tcells
(Moskophidis *et al.* 1987: Lehmann-Grube *et* similar outcome to that obtained in completely T-cell-defi-
cient mice is to be found in mice deficient of $CD8^+$ Tcells
(Moskophidis *et al.* 1987; Lehmann-Grube *et al.* 1993;
Christensen *et al.* 1994) This fact, togeth cient mice is to be found in mice deficient of CD8⁺ Tcells (Moskophidis *et al.* 1987; Lehmann-Grube *et al.* 1993; Christensen *et al.* 1994). This fact, together with the much older finding that virus control correlate (Moskophidis *et al.* 1987; Lehmann-Grube *et al.* 1993; Christensen *et al.* 1994). This fact, together with the much older finding that virus control correlates with cytotoxic Christensen *et al.* 1994). This fact, together with the much
older finding that virus control correlates with cytotoxic
T-lymphocyte (CTL) activity *ex vivo* (Marker & Volkert
1973) for many vears focused the attention en older finding that virus control correlates with cytotoxic

T-lymphocyte (CTL) activity ex vivo (Marker & Volkert

1973), for many years focused the attention entirely on

virus-specific CTI s as the crucial effector subse T-lymphocyte (CTL) activity *ex vivo* (Marker & Volkert 1973), for many years focused the attention entirely on virus-specific CTLs as the crucial effector subset.
Although still correct in its essence—and indeed further 1973), for many years focused the attention entirely on
virus-specific CTLs as the crucial effector subset.
Although still correct in its essence—and indeed further
confirmed by the inability of perforinvirus-specific CTLs as the crucial effector subset.
Although still correct in its essence—and indeed further confirmed by the inability of perforin-
deficient mice to control this infection (Walsh *et al.* 1994; Although still correct in its essence—and indeed further confirmed by the inability of perforin-
deficient mice to control this infection (Walsh *et al.* 1994;
Kagi *et al.* 1994)—recent studies by several groups,
including our own have revealed a much more complex deficient mice to control this infection (Walsh *et al.* 1994;
Kagi *et al.* 1994)—recent studies by several groups,
including our own, have revealed a much more complex
picture of the virus-bost interplay Kagi *et al.* 1994)—recent studies
including our own, have revealed a
picture of the virus-host interplay.
In this report we have focused including our own, have revealed a much more complex
picture of the virus-host interplay.
In this report we have focused on two critical issues

picture of the virus–host interplay.
In this report we have focused on two critical issues
relevant to the general understanding of T-cell-mediated
antiviral immunity. First, how is antiviral CD8⁺ T-cell In this report we have focused on two critical issues
relevant to the general understanding of T-cell-mediated
antiviral immunity. First, how is antiviral CD8⁺ T-cell
activity maintained thus preventing the virus from relevant to the general understanding of T-cell-mediated
antiviral immunity. First, how is antiviral CD8⁺ T-cell
activity maintained, thus preventing the virus from vir
regaining a positive growth rate subsequent to the antiviral immunity. First, how is antiviral CDB^+ T-cell activity maintained, thus preventing the virus from regaining a positive growth rate subsequent to the initial activity maintained, thus preventing the virus from
regaining a positive growth rate subsequent to the initial
phase of virus control. Second, what is the importance of
different molecular effector systems in the normal fu regaining a positive growth rate subsequent to the initial
phase of virus control. Second, what is the importance of
different molecular effector systems in the normal func-
tioning of virus-specific CTLs in vine phase of virus control. Second, what is the importance of
different molecular effector systems in the normal func-
tioning of virus-specific CTLs *in vivo*.
Essential to our analysis of the above issues has been
the use of

tioning of virus-specific CTLs in vivo.

Essential to our analysis of the above issues has been

the use of virus strains that differ in their capacity to

replicate and spread in the bost Various strains of LCMV Essential to our analysis of the above issues has been (
the use of virus strains that differ in their capacity to
replicate and spread in the host. Various strains of LCMV w
may be classified according to their ability to replicate and spread in the host. Various strains of LCMV may be classified according to their ability to rapidly replicate and spread in the host. Various strains of LCMV
may be classified according to their ability to rapidly
attain high titres in the viscera following intravenous or
intraneritoneal infection and previous studies ha may be classified according to their ability to rapidly
attain high titres in the viscera following intravenous or
intraperitoneal infection, and previous studies have
demonstrated a clear correlation between this ability attain high titres in the viscera following intravenous or
intraperitoneal infection, and previous studies have
demonstrated a clear correlation between this ability and
the canacity (at high doses of infection) to establi intraperitoneal infection, and previous studies have
demonstrated a clear correlation between this ability and
the capacity (at high doses of infection) to establish
chronic infection in adult mice (Moskophidis et al. 1994 demonstrated a clear correlation between this ability and
the capacity (at high doses of infection) to establish
chronic infection in adult mice (Moskophidis *et al.* 1994*a*,
1995; King *et al.* 1990; Pfau *et al.* 1982). chronic infection in adult mice (Moskophidis *et al.* 1994*a*, 1995; King *et al.* 1990; Pfau *et al.* 1982). The chronic infection is generally associated with at least some degree of 1995; King *et al.* 1990; Pfau *et al.* 1982). The chronic infection is generally associated with at least some degree of immune exhaustion, i.e. anergy or actual depletion of relevant T -cell populations obtained throug tion is generally associated with at least some degree of
immune exhaustion, i.e. anergy or actual depletion of
relevant T-cell populations obtained through extended
exhaustive stimulation with antigen (Moskophidis *et al.* immune exhaustion, i.e. anergy or actual depletion of
relevant T-cell populations obtained through extended
exhaustive stimulation with antigen (Moskophidis *et al.*
1993–1994–1995: Ahmed & Oldstone 1988: Thomsen & relevant T-cell populations obtained through extended expansion and differentiation did not critically depend on exhaustive stimulation with antigen (Moskophidis *et al.* CD4⁺ T-cell help. This conclusion was further su exhaustive stimulation with antigen (Moskophidis et al.

neonatally infected mice may survive for the normal life-
train with limited capacity to persist even following
time of a mouse as persistent virus carriers, and such
early acquired infections probably constitute the most
 In our own studies we have been using two LCMV
rains representing both ends of this spectrum. The In our own studies we have been using two LCMV
strains representing both ends of this spectrum. The
LCMV Armstong strain represents a pop-viscerotropic In our own studies we have been using two LCMV
strains representing both ends of this spectrum. The
LCMV Armstong strain represents a non-viscerotropic
strain with limited canacity to persist even following strains representing both ends of this spectrum. The LCMV Armstong strain represents a non-viscerotropic
strain with limited capacity to persist even following
inoculation with extreme doses of virus (Moskophidis LCMV Armstong strain represents a non-viscerotropic
strain with limited capacity to persist even following
inoculation with extreme doses of virus (Moskophidis
 $et \ al \ 1994a \ 1995$) The Traub strain on the other hand strain with limited capacity to persist even following spreads rapidly to a number of organ sites and replicates *et al.* 1994*a*, 1995). The Traub strain on the other hand
spreads rapidly to a number of organ sites and replicates
to high titres (Moskophidis *et al.* 1994*b*; Thomsen &
Marker 1989: Thomsen *et al.* 1998*b*) Moreover, spreads rapidly to a number of organ sites and replicates
to high titres (Moskophidis *et al.* 1994*b*; Thomsen &
Marker 1989; Thomsen *et al.* 1998*b*). Moreover, chronic
infection is easily induced as evidenced by the fi Marker 1989; Thomsen *et al.* 1998b). Moreover, chronic infection is easily induced as evidenced by the finding Marker 1989; Thomsen *et al.* 1998*b*). Moreover, chronic infection is easily induced as evidenced by the finding that a 100-fold increase in inoculum may change the course of infection with this strain from an acute type infection is easily induced as evidenced by the finding
that a 100-fold increase in inoculum may change the
course of infection with this strain from an acute type to
a chronic pattern (Marker *et al.* 1985; Marker & that a 100-fold increase in inoculum may change the course of infection with this strain from an acute type to a chronic pattern (Marker *et al.* 1985; Marker & Thomsen 1987) course of infection with this strain from an acute type to
a chronic pattern (Marker *et al.* 1985; Marker &
Thomsen 1987).

3. THE IMPORTANCE OF CD4⁺ HELP IN MAINTAINING EFFICIENT CD8⁺ T-CELL SURVEILLANCE IN PERSISTENT VIRAL INFECTION
IN PERSISTENT VIRAL INFECTION

While $CD8⁺$ T cells are central effectors in the control of LCMV infection, initial studies failed to reveal an While CDB^+ T cells are central effectors in the control
of LCMV infection, initial studies failed to reveal an
essential role for $CD4^+$ T helper cells in determining the
outcome of this infection (Moskophidis *et al.* of LCMV infection, initial studies failed to reveal an essential role for CD4⁺ T helper cells in determining the outcome of this infection (Moskophidis *et al.* 1987; Ahmed *et al.* 1988: Christensen *et al.* 1994) Thus essential role for CD4⁺ T helper cells in determining the outcome of this infection (Moskophidis *et al.* 1987; Ahmed *et al.* 1988; Christensen *et al.* 1994). Thus, both acute (treatment with monoclonal antibodies) and outcome of this infection (Moskophidis *et al.* 1987; Ahmed *et al.* 1988; Christensen *et al.* 1994). Thus, both acute (treatment with monoclonal antibodies) and chronic (major histocompatibility complex (MHC) class II defi-(treatment with monoclonal antibodies) and chronic
(major histocompatibility complex (MHC) class II defi-
ciency) depletion of $CD4^+$ T cells failed to influence the
magnitude of the CTL response and the efficiency of (major histocompatibility complex (MHC) class II deficiency) depletion of $CD4^+$ T cells failed to influence the magnitude of the CTL response and the efficiency of virus control *in vine*. However, the original conclusio ciency) depletion of CD4⁺ T cells failed to influence the
magnitude of the CTL response and the efficiency of
virus control *in vivo*. However, the original conclusions
were based exclusively on analysis of the acute pha magnitude of the CTL response and the efficiency of virus control *in vivo*. However, the original conclusions were based exclusively on analysis of the acute phase of the infection i.e. up to around day 10 post-infection virus control *in vivo*. However, the original conclusions were based exclusively on analysis of the acute phase of the infection, i.e. up to around day 10 post-infection (p.i.). Furthermore, experiments were initially co were based exclusively on analysis of the acute phase of the infection, i.e. up to around day 10 post-infection (p.i.). Furthermore, experiments were initially conducted with slowly invasive virus strains only. the infection, i.e. up to around day 10 post-infection (p.i.). rthermore, experiments were initially conducted with
wly invasive virus strains only.
Subsequent analysis by several groups revealed that in
 Ω 4⁺-deficient mice, infections with viscerotronic strains

 $CD4^+$ -de -
invasive virus strains only.
sequent analysis by several groups revealed that in
-deficient mice, infections with viscerotropic strains
MV resulted in failure to control the infection and Subsequent analysis by several groups revealed that in CD4⁺-deficient mice, infections with viscerotropic strains of LCMV resulted in failure to control the infection and to establish efficient long-term CTI memory thus $CD4^+$ -deficient mice, infections with viscerotropic strains
of LCMV resulted in failure to control the infection and
to establish efficient long-term CTL memory, thus
revealing a critical role for $CD4^+$ Teells under con of LCMV resulted in failure to control the infection and
to establish efficient long-term CTL memory, thus
revealing a critical role for $CD4^+$ T cells under conditions
of chronic Leell stimulation (Battegay *et al* 1994: to establish efficient long-term CTL memory, thus
revealing a critical role for CD4⁺ T cells under conditions
of chronic T-cell stimulation (Battegay *et al.* 1994; Matlou-
bian *et al.* 1994). While these results were g revealing a critical role for CD4⁺ T cells under conditions of chronic T-cell stimulation (Battegay *et al.* 1994; Matloubian *et al.* 1994). While these results were generally of chronic T-cell stimulation (Battegay *et al.* 1994; Matlou-
bian *et al.* 1994). While these results were generally
obtained using relatively high virus doses for inoculation,
our own studies employing a relatively low bian *et al.* 1994). While these results were generally obtained using relatively high virus doses for inoculation, our own studies employing a relatively low dose of the viscerotronic Traub strain allowed a more precise obtained using relatively high virus doses for inoculation,
our own studies employing a relatively low dose of the
viscerotropic Traub strain allowed a more precise defini-
tion of the role played by $CD4^+$ Tcells in the our own studies employing a relatively low dose of the viscerotropic Traub strain allowed a more precise definition of the role played by CD4⁺ Tcells in the anti-LCMV response. Thus by studying MHC class II-deficient mice tion of the role played by CD4⁺ Tcells in the anti-LCMV
response. Thus by studying MHC class II-deficient mice
infected with *ca*. 200 plaque-forming units (pfu) of
LCMV Traub a binhasic response pattern was disclosed response. Thus by studying MHC class II-deficient mice
infected with *ca*. 200 plaque-forming units (pfu) of
LCMV Traub, a biphasic response pattern was disclosed
(Christensen *et al* 1994: Thomsen *et al* 1996) infected with *ca.* 200 plaque-forming unit:
LCMV Traub, a biphasic response pattern wa
(Christensen *et al.* 1994; Thomsen *et al.* 1996).
Under these conditions the primary CTI LCMV Traub, a biphasic response pattern was disclosed (Christensen *et al.* 1994; Thomsen *et al.* 1996).
Under these conditions, the primary CTL response

was unimpaired—except for a slightly premature decline of activity in some mice—and transient virus control was was unimpaired—except for a slightly premature decline
of activity in some mice—and transient virus control was
observed. However, after about two months CTL
memory could not be demonstrated, and at about the of activity in some mice—and transient virus control was
observed. However, after about two months CTL
memory could not be demonstrated, and at about the
same time bigh titres of virus could be detected in the observed. However, after about two months CTL
memory could not be demonstrated, and at about the
same time high titres of virus could be detected in the
blood as well as in several organs. Hence, it appeared memory could not be demonstrated, and at about the
same time high titres of virus could be detected in the
blood as well as in several organs. Hence, it appeared
that CD4⁺ T cells were somehow essential to the longsame time high titres of virus could be detected in the blood as well as in several organs. Hence, it appeared that $CD4^+$ T cells were somehow essential to the long-term maintenance of virus-specific $CD8^+$ T-cell activi blood as well as in several organs. Hence, it appeared
that $CD4^+$ T cells were somehow essential to the long-
term maintenance of virus-specific $CD8^+$ T-cell activity
and immune surveillance in mice infected with rapidl that $CD4^+$ T cells were somehow essential to the long-
term maintenance of virus-specific $CD8^+$ T-cell activity
and immune surveillance in mice infected with rapidly term maintenance of virus-specific CD8⁺ T-cell activity
and immune surveillance in mice infected with rapidly
replicating virus strains, whereas the initial CD8⁺ T-cell
expansion and differentiation did not critically and immune surveillance in mice infected with rapidly
replicating virus strains, whereas the initial CD8⁺ T-cell
expansion and differentiation did not critically depend on
CD4⁺ T-cell belp. This conclusion was further replicating virus strains, whereas the initial $CD8^+$ T-cell
expansion and differentiation did not critically depend on
 $CD4^+$ T-cell help. This conclusion was further supported
by the finding that transient $CD4^+$ T-cell expansion and differentiation did not critically depend on
CD4⁺ T-cell help. This conclusion was further supported
by the finding that transient CD4⁺ T-cell depletion
during the acute response had little impact on the $CD4^+$ T-cell help. This conclusion was further supported
by the finding that transient $CD4^+$ T-cell depletion
during the acute response had little impact on the final

time after infection
Figure 1. Frequency of LCMV-specific CD8⁺ T cells in LCMV Armstrong-infected B-cell deficient (µMT/µMT) and wild-type
(wMT/+) mice as a function of time. Mice were infected intravenously with 4800 pl Figure 1. Frequency of LCMV-specific CD8⁺ T cells in LCMV Armstrong-infected B-cell deficient (μ MT/ μ MT) and wild-type (μ MT/+) mice as a function of time. Mice were infected intravenously with 4800 plaque-formin Figure 1. Frequency of LCMV-specific CD8⁺ T cells in LCMV Armstrong-infected B-cell deficient (μ MT/ μ MT) and wild-type (μ MT/+) mice as a function of time. Mice were infected intravenously with 4800 plaque-formin $(\mu MT)^{+}$) mice as a function of time. Mice were infected intravenously with 4800 plaque-forming units of LCMV Armstron
and on the indicated days (a) GP33-41- and (b) NP396-404-specific CD8⁺ T cells were visualized thro and on the indicated days (a) GP33-41- and (b) NP396-404-specific CD8⁺ T cells were visualized through detection of IFN- γ intracellularly (for details see Murali-Krishna *et al.* 1998 and legend to figure 2). Columns intracellularly (for details see Murali-Krishna *et al.* 1998 and legend to figure 2). Columns show the frequency of peptide
specific CD8⁺ T cells per total CD8⁺ T cells (median and ranges of two to 13 mice). Figures a specific CD8⁺ T cells per total CD8⁺ T cells (median and ranges of two to 13 mice).
fluorescence intensity of cells with regard to intracellular staining for IFN- γ ; mean f
two strains during the acute response (see

two strains during the acute response (see figure 2 for representative outcome of infection (Thomsen *et al.* 1996), except when very high doses of viscerotropic virus were inoculated outcome of infection (Thomsen *et al.* 1996), except when
very high doses of viscerotropic virus were inoculated
(Matloubian *et al.* 1994) outcome of infection (The
very high doses of visce:
(Matloubian *et al*. 1994).

**4. THE ANTIVIRAL CD8⁺ T-CELL RESPONSE INTIVIRAL CD8⁺ T-CELL RESPO
IN B-CELL-DEFICIENT MICE**

4. THE ANTIVIAAL CD6 T-CELL RESPONSE

IN B-CELL-DEFICIENT MICE

One important function of $CD4^+$ T cells is to help in

cell activation and since neutralizing antibodies might \blacksquare

Decell activation, and since neutralizing antibodies might
 \blacksquare are neutralizing antibodies might
 \blacksquare are neutralizing antibodies might One important function of $CD4^+$ T cells is to help in B-cell activation, and since neutralizing antibodies might play a role in reducing the viral load that might other-wise exhaust the $CD8^+$ T-cell population through c B-cell activation, and since neutralizing antibodies might
play a role in reducing the viral load that might other-
wise exhaust the $CD8^+$ T-cell population through chronic
stimulation (Thomsen & Marker 1988: Battegay *e* play a role in reducing the viral load that might otherwise exhaust the CD8⁺ T-cell population through chronic stimulation (Thomsen & Marker 1988; Battegay *et al.* 1993) we next analysed the response to LCMV infection wise exhaust the $CD8^+$ T-cell population through chronic Traub-infected mice. Thus, CTL memory was reduced stimulation (Thomsen & Marker 1988; Battegay *et al.* only about fourfold, as demonstrated under bulk condi-
1993 stimulation (Thomsen & Marker 1988; Battegay *et al.* 1993), we next analysed the response to LCMV infection in B-cell-deficient mice. Using infection with *ca*. 200 pfu of LCMV Traub, we observed a nattern not much dis-1993), we next analysed the response to LCMV infection
in B-cell-deficient mice. Using infection with ca . 200 pfu
of LCMV Traub, we observed a pattern not much dis-
similar to that observed in MHC class IL-deficient mice in B-cell-deficient mice. Using infection with ca . 200 pfu
of LCMV Traub, we observed a pattern not much dis-
similar to that observed in MHC class II-deficient mice:
the initial CTI response was only slightly impaired a of LCMV Traub, we observed a pattern not much dis-
similar to that observed in MHC class II-deficient mice: Ahmed 1996).
the initial CTL response was only slightly impaired and Based on the
transient virus control was obse similar to that observed in MHC class II-deficient mice:

e FACS plots).
infected B-cell-deficient mice (Thomsen *et al.* 1996).
However also in these mice we eventually noted a breakinfected B-cell-deficient mice (Thomsen *et al.* 1996).
However, also in these mice we eventually noted a break-
down of virus control and failure to detect CTI memory infected B-cell-deficient mice (Thomsen *et al.* 1996).
However, also in these mice we eventually noted a break-
down of virus control and failure to detect CTL memory.
In contrast, B-cell-deficient mice infected with an e However, also in these mice we eventually noted a break-
down of virus control and failure to detect CTL memory.
In contrast, B-cell-deficient mice infected with an even
higher dose of the slowly replicating LCMV Armstrong down of virus control and failure to detect CTL memory.
In contrast, B-cell-deficient mice infected with an even
higher dose of the slowly replicating LCMV Armstrong
strain were able to almost eliminate the virus (Asano & In contrast, B-cell-deficient mice infected with an even
higher dose of the slowly replicating LCMV Armstrong
strain were able to almost eliminate the virus (Asano &
Ahmed 1996: Thomsen *et al.* 1996), and only low levels higher dose of the slowly replicating LCMV Armstrong
strain were able to almost eliminate the virus (Asano &
Ahmed 1996; Thomsen *et al.* 1996), and only low levels strain were able to almost eliminate the virus (Asano & Ahmed 1996; Thomsen *et al.* 1996), and only low levels could be detected primarily in the lungs after four to five months (Thomsen *et al.* 1998*b*). Also with rega Ahmed 1996; Thomsen *et al.* 1996), and only low levels
could be detected primarily in the lungs after four to five
months (Thomsen *et al.* 1998*b*). Also with regard to main-
taining CTI surveillance, the latter mice dif could be detected primarily in the lungs after four to five
months (Thomsen *et al.* 1998*b*). Also with regard to main-
taining CTL surveillance, the latter mice differed from
Traub-infected mice. Thus, CTL memory, was re months (Thomsen *et al.* 1998*b*). Also with regard to maintaining CTL surveillance, the latter mice differed from
Traub-infected mice. Thus, CTL memory was reduced
only about fourfold as demonstrated under bulk conditaining CTL surveillance, the latter mice differed from
Traub-infected mice. Thus, CTL memory was reduced
only about fourfold, as demonstrated under bulk condi-
tions (A. R. Thomsen, unpublished observation) and Traub-infected mice. Thus, CTL memory was reduced
only about fourfold, as demonstrated under bulk condi-
tions (A. R. Thomsen, unpublished observation) and
analysis of cytotoxic T-lymphocyte precursors (CTLp) analysis of cytotoxic T-lymphocyte precursors (CTLp) tions (A. R. Thomsen, unpublished observation) and
analysis of cytotoxic T-lymphocyte precursors (CTLp)
frequencies yielded essentially similar results (Asano &
Ahmed 1996) analysis of cyte
frequencies yiel
Ahmed 1996).
Based on the equencies yielded essentially similar results (Asano &
amed 1996).
Based on these findings, we concluded that the main
paction of CD4⁺ T cells was to belp B cells, and that the

Based on these findings, we concluded that the main function of CD4⁺ T cells was to help B cells, and that the

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VLA-4 (log fluorescence intensity)

Figure 2. Impaired functional capacity of LCMV-specific CD8⁺ memory T cells in B-cell-deficient mice. B-cell-deficient Figure 2. Impaired functional capacity of LCMV-specific CD8⁺ memory T cells in B-cell-deficient mice. B-cell-deficient (μ MT/ μ MT) and wild-type (μ MT/+) mice were infected with 4800 plaque-forming units intravenou Figure 2. Impaired functional capacity of LCMV-specific CD8⁺ memory T cells in B-cell-deficient mice. B-cell-deficient $(\mu MT/\mu MT)$ and wild-type $(\mu MT/+)$ mice were infected with 4800 plaque-forming units intravenously, and $(\mu MT/\mu MT)$ and wild-type $(\mu MT/+)$ mice were infected with 4800 plaque-forming units intravenously, and on day (*a*) 10
and (*b*) 125 post-infection splenocytes were harvested and stimulated with GP33-41 and NP396-404 for 5 and (b) 125 post-infection splenocytes were harvested and stimulated with GP33-41 and NP396-404 for 5 h in the presence
of monensin. Following *in vitro* incubation, cells were surface labelled with anti-CD8 and anti-VLAof monensin. Following *in vitro* incubation, cells were surface labelled with anti-CD8 and anti-VLA-4 (activation marker),
permeabilized and stained with anti-IFN-y. Gates have been set for CD8⁺ T cells, representative

depicted. Only results for NP396-404-specific cells are shown, l
lack of antibodies leads to an increased virus load, even-
tually resulting in exhaustion of the CD8⁺ subset lack of antibodies leads to an increased virus load, eventually resulting in exhaustion of the CDB^+ subset (Thomsen *et al* 1996). However more recent studies have lack of antibodies leads to an increased virus load, eventually resulting in exhaustion of the CD8⁺ subset (Thomsen *et al.* 1996). However, more recent studies have pointed towards a possible role of B cells as critical tually resulting in exhaustion of the $CD8^+$ subset
(Thomsen *et al.* 1996). However, more recent studies have
pointed towards a possible role of B cells as critical modi-
fiers of $CD4^+$ and $CD8^+$ activity perhaps in th (Thomsen *et al.* 1996). However, more recent studies have
pointed towards a possible role of B cells as critical modi-
fiers of $CD4^+$ and $CD8^+$ activity perhaps in the role as
antigen-presenting cells. Thus Homann et a pointed towards a possible role of B cells as critical modi-
fiers of $CD4^+$ and $CD8^+$ activity perhaps in the role as
antigen-presenting cells. Thus, Homann et al. (1998) have
presented evidence indicating that the $CD4^$ fiers of $CD4^+$ and $CD8^+$ activity perhaps in the role as antigen-presenting cells. Thus, Homann et al. (1998) have presented evidence indicating that the $CD4^+$ and $CD8^+$ T cells primed and present in LCMV-infected B-c presented evidence indicating that the CD4⁺ and CD8⁺
T cells primed and present in LCMV-infected B-cell-
deficient mice are qualitatively inferior to those primed
and maintained in the presence of B cells T cells primed and present in LCMV-in
deficient mice are qualitatively inferior to
and maintained in the presence of B cells.
To study this in greater detail, we ficient mice are qualitatively inferior to those primed
d maintained in the presence of B cells.
To study this in greater detail, we analysed the
petics of the virus-specific $CD8^+$ T-cell response in

and maintained in the presence of B cells.

To study this in greater detail, we analysed the

kinetics of the virus-specific $CD8^+$ T-cell response in
 R_{cell} -deficient mice infected with 4800 pfu of LCMV To study this in greater detail, we analysed the
kinetics of the virus-specific CD8⁺ T-cell response in
B-cell-deficient mice infected with 4800 pfu of LCMV
Armstrong visualizing antigen-specific cells through in B-cell-deficient mice infected with 4800 pfu of LCMV
Armstrong, visualizing antigen-specific cells through
detection of intracellular interferon- γ (IFN- γ). By this
annroach we found that while the initial response t Armstrong, visualizing antigen-specific cells through
detection of intracellular interferon- γ (IFN- γ). By this
approach we found that while the initial response tends
to be slightly delayed at least the same frequen detection of intracellular interferon- γ (IFN- γ). By this
approach we found that while the initial response tends
to be slightly delayed, at least the same frequency of
LCMV-enecific CD⁸⁺ T cells are generated in B approach we found that while the initial response tends
to be slightly delayed, at least the same frequency of
LCMV-specific CDB^+ T cells are generated in B-cell-

mular results have been obtained for GP33-41-specific cells.
deficient mice (figure 1), although the absolute number
is lower due to the reduced size of secondary lymphoid deficient mice (figure 1), although the absolute number
is lower due to the reduced size of secondary lymphoid
organs in B-cell-deficient mice. However, as time passes deficient mice (figure 1), although the absolute number
is lower due to the reduced size of secondary lymphoid
organs in B-cell-deficient mice. However, as time passes,
the LCMV-specific CD8⁺ T cells in B-cell-deficient is lower due to the reduced size of secondary lymphoid
organs in B-cell-deficient mice. However, as time passes,
the LCMV-specific CD8⁺ T cells in B-cell-deficient mice
become qualitatively inferior to those in infected organs in B-cell-deficient mice. However, as time passes,
the LCMV-specific CD8⁺ T cells in B-cell-deficient mice
become qualitatively inferior to those in infected wild-
type mice as evidenced by lower mean fluorescence the LCMV-specific $CD8^+$ T cells in B-cell-deficient mice
become qualitatively inferior to those in infected wild-
type mice, as evidenced by lower mean fluorescence
following staining for IFN- χ (figures 1 and 2). In o become qualitatively inferior to those in infected wild-
type mice, as evidenced by lower mean fluorescence
following staining for IFN- γ (figures 1 and 2). In other
studies we have found that this is a relevant paramete type mice, as evidenced by lower mean fluorescence following staining for IFN- γ (figures 1 and 2). In other studies we have found that this is a relevant parameter for the capacity of the Tcells to secrete IFN- γ . following staining for IFN- γ (figures 1 and 2). In other studies we have found that this is a relevant parameter for the capacity of the Tcells to secrete IFN- γ .
In continuation of this finding, we analysed CD8⁺ T

kinetics of the virus-specific $CD8^+$ T-cell response in that more memory $(CD44^{\text{high}})$ $CD8^+$ T cells in B-cell-
B-cell-deficient mice infected with 4800 pfu of LCMV deficient mice were actively cycling for months after
 the capacity of the Tcells to secrete IFN- γ .
In continuation of this finding, we analysed CD8⁺ T-cell
turnover and phenotype in both groups of mice, and found
that more memory (CD44^{high}), CD8⁺ T cells in B-cell-In continuation of this finding, we analysed $CD8^+$ T-cell
turnover and phenotype in both groups of mice, and found
that more memory $(CD44^{\text{high}})$ $CD8^+$ T cells in B-cell-
deficient mice, were, actively, cycling for mont turnover and phenotype in both groups of mice, and found
that more memory (CD44^{high}) CD8⁺ T cells in B-cell-
deficient mice were actively cycling for months after
infection as revealed by increased BrdU incorporation that more memory $(CD44^{high})$ $CD8^+$ T cells in B-celldeficient mice were actively cycling for months after
infection, as revealed by increased BrdU incorporation
over a seven-day pulse period. Furthermore, a significantly
higher fraction of the CD8⁺ Tcells in B-cell-defici infection, as revealed by increased BrdU incorporation
over a seven-day pulse period. Furthermore, a significantly
higher fraction of the CD8⁺ Tcells in B-cell-deficient mice
maintained an L-selectin^{low} phenotype, and over a seven-day pulse period. Furthermore, a significantly
higher fraction of the CD8⁺ Tcells in B-cell-deficient mice
maintained an L-selectin^{low} phenotype, and this subset
comprised the maiority of cycling CD8⁺ Tc higher fraction of the $CD8^+$ T cells in B-cell-deficient mice
maintained an L-selectin^{low} phenotype, and this subset
comprised the majority of cycling $CD8^+$ T cells (figure 3);

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(a) day 8, (b) day 80, (c) day 125 and (d) approximately day 240 post-infection.
in wild-type mice a similar pattern is observed only during infected mic Figure 3. Permanently increased CD8⁺ T cell turnover in LCMV Armstong-infected B-cell-deficient mice. B-cell-deficient and wild-type mice were infected with 4800 plaque-forming units of LCMV Armstrong intravenously, and for a period of either three Figure 3. Permanently increased CD8⁺ T cell turnover in LCMV Armstong-infected B-cell-deficient mice. B-cell-deficient and
wild-type mice were infected with 4800 plaque-forming units of LCMV Armstrong intravenously, and wild-type mice were infected with 4800 plaque-forming units of LCMV Armstrong intravenously, and for a period of either three
(acute) or seven (memory) days prior to analysis, mice were given BrdU in their drinking water t (acute) or seven (memory) days prior to analysis, mice were given BrdU in their drinking water to label *in vivo* dividing cells. On
the indicated days, splenocytes were harvested and cells were surface labelled with anti the indicated days, splenocytes were harvested and cells were surface labelled with anti-CD8 and anti-L-selectin, permeabilized
and stained with anti-BrdU. Gates have been set for CD8⁺ T cells, and representative results

in wild-type mice a similar pattern is observed only during
the acute response. Consequently this finding points to
opgoing $C\text{D}8^+$ T-cell activation in B-cell-deficient mice in wild-type mice a similar pattern is observed only during
the acute response. Consequently this finding points to
ongoing CD8⁺ T-cell activation in B-cell-deficient mice.
Overall, these findings confirm and extend the e acute response. Consequently this finding points to
going CD8⁺ T-cell activation in B-cell-deficient mice.
Overall, these findings confirm and extend the conclu-
on that the CD8⁺ subset behaves very differently in

ongoing CD8⁺ T-cell activation in B-cell-deficient mice.
Overall, these findings confirm and extend the conclu-
sion that the CD8⁺ subset behaves very differently in
B-cell-deficient mice compared with similarly infect Overall, these findings confirm and extend the conclusion that the CD8⁺ subset behaves very differently in B-cell-deficient mice compared with similarly infected wild-types. Notably, the lower per cell capacity to sion that the $CD8^+$ subset behaves very differently in B-cell-deficient mice compared with similarly infected wild-types. Notably, the lower per cell capacity to produce IFN- γ suggests that the LCMV-specific $CD8^+$ T B-cell-deficient mice compared with similarly infected Under normal conditions, naive T cells require two wild-types. Notably, the lower per cell capacity to signals for activation (Davidson 1977; Lafferty & produce IFNwild-types. Notably, the lower per cell capacity to produce IFN- γ suggests that the LCMV-specific CD8⁺ T cells present in the former mice are partially anergized (Zajac *et al.* 1998). This together with the evidence produce IFN- γ suggests that the LCMV-specific CD8⁺ T cells present in the former mice are partially anergized (Zajac *et al.* 1998). This, together with the evidence for chronic T-cell activation observed in B-cell-d cells present in the former mice are partially anergized (Zajac *et al.* 1998). This, together with the evidence for chronic T-cell activation observed in B-cell-deficient mice suggests that in the absence of B cells the (Zajac *et al.* 1998). This, together with the evidence for chronic T-cell activation observed in B-cell-deficient mice, suggests that in the absence of B cells, the $CD8⁺$ cell subset in LCMV-infected mice is subject to chronic mice, suggests that in the absence of B cells, the CD8⁺
cell subset in LCMV-infected mice is subject to chronic
stimulation leading to partial anergy. Since the virus load
in LCMV Armstrong-infected B-cell-deficient mice cell subset in LCMV-infected mice is subject to chronic
stimulation leading to partial anergy. Since the virus load
in LCMV Armstrong-infected B-cell-deficient mice
remains limited for most of the observation period stimulation leading to partial anergy. Since the virus load
in LCMV Armstrong-infected B-cell-deficient mice
remains limited for most of the observation period
(Thomsen *et al.* 1998b) an indirect effect reflection the in LCMV Armstrong-infected B-cell-deficient mice
remains limited for most of the observation period
(Thomsen *et al.* 1998*b*), an indirect effect reflecting the
lack of an antibody-mediated reduction in the viral load remains limited for most of the observation period (Thomsen *et al.* 1998*b*), an indirect effect reflecting the lack of an antibody-mediated reduction in the viral load constitutes an unlikely mechanism. The presence of (Thomsen *et al.* 1998*b*), an indirect effect reflecting the lack of an antibody-mediated reduction in the viral load constitutes an unlikely mechanism. The presence of a normal B-cell population must therefore in itself lack of an antibody-mediated reduction in the viral load
constitutes an unlikely mechanism. The presence of a
normal B-cell population must therefore in itself provide
an environment essential to the normal functioning of constitutes an unlikely mechanism. The presence of a
normal B-cell population must therefore in itself provide
an environment essential to the normal functioning of
 CDR^+ T cells in virus infected mice. Therefore, conclunormal B-cell population must therefore in itself provide
an environment essential to the normal functioning of
CD8⁺ T cells in virus-infected mice. Therefore, conclu-
sions based on results obtained in B-cell-deficient an environment essential to the normal functioning of $CD8^+$ T cells in virus-infected mice. Therefore, conclusions based on results obtained in B-cell-deficient mice need to be interpreted with great care. Indeed, similar CDB^+ T cells in virus-infected mice. Therefore, conclusions based on results obtained in B-cell-deficient mice
need to be interpreted with great care. Indeed, similar
indirect mechanisms, may explain recent findings in sions based on results obtained in B-cell-deficient mice
need to be interpreted with great care. Indeed, similar
indirect mechanisms may explain recent findings in
several autoimmune models implicating B cells in the need to be interpreted with great care. Indeed, similar
indirect mechanisms may explain recent findings in
several autoimmune models implicating B cells in the
pathogenesis despite lack of any other evidence pointing indirect mechanisms may explain recent findings in several autoimmune models implicating B cells in the pathogenesis, despite lack of any other evidence pointing several autoimmune models implicating B cells in the pathogenesis, despite lack of any other evidence pointing to antibody-mediated effector mechanisms (Serreze *et al.* 1996: Wolf *et al.* 1996: Chan *et al.* 1999) pathogenesis, despite lack of any other *e*to antibody-mediated effector mechanis
1996; Wolf *et al.* 1996; Chan *et al.* 1999).

5. ROLE OF CO-STIMULATION IN THE MAINTENANCE OF CD8⁺ T-CELL MEDIATED IMMUNITY OF CD8⁺ T-CELL MEDIATED IMMUNITY
Another approach to study the mechanisms underlying

the maintenance of $CD8⁺$ effector capacity in persistently

infected mice is to search for critical molecular interinfected mice is to search for critical molecular inter-
actions. In this context, a number of studies have pointed
to CD40-CD40 ligand as a set of molecules central in the infected mice is to search for critical molecular inter-
actions. In this context, a number of studies have pointed
to CD40-CD40 ligand as a set of molecules central in the
control of T-cell activation (for a review see G actions. In this context, a number of studies have pointed
to CD40–CD40 ligand as a set of molecules central in the
control of T-cell activation (for a review see Grewal &
Elavell 1998: Laman et al. 1996) to CD40-CD40 ligand as a set of molecules central in the control of T-cell activation (for a review see Grewal & Flavell 1998; Laman *et al.* 1996).

Under normal conditions, naive T cells require two Flavell 1998; Laman *et al.* 1996).

Under normal conditions, naive T cells require two

signals for activation (Davidson 1977; Lafferty &

Cunningham 1975) The first is delivered through T-cell Under normal conditions, naive T cells require two
signals for activation (Davidson 1977; Lafferty &
Cunningham 1975). The first is delivered through T-cell
receptor binding to relevant pentide–MHC complexes on signals for activation (Davidson 1977; Lafferty &
Cunningham 1975). The first is delivered through T-cell
receptor binding to relevant peptide–MHC complexes on
the surface of antigen-presenting cells (APCs). The second Cunningham 1975). The first is delivered through T-cell
receptor binding to relevant peptide–MHC complexes on
the surface of antigen-presenting cells (APCs). The second
signal is provided through interaction with various m receptor binding to relevant peptide–MHC complexes on
the surface of antigen-presenting cells (APCs). The second
signal is provided through interaction with various mol-
ecules presented on the surface of professional APCs the surface of antigen-presenting cells (APCs). The second
signal is provided through interaction with various mol-
ecules presented on the surface of professional APCs. A
pre-condition for efficient delivery of the second signal is provided through interaction with various molecules presented on the surface of professional APCs. A
pre-condition for efficient delivery of the second signal,
commonly known as co-stimulation, is previous activa ecules presented on the surface of professional APCs. A
pre-condition for efficient delivery of the second signal,
commonly known as co-stimulation, is previous activa-
tion of the APC (Liu & Janeway Ir 1991: Banchereau & pre-condition for efficient delivery of the second signal, commonly known as co-stimulation, is previous activation of the APC (Liu & Janeway Jr 1991; Banchereau & Steinman 1998). commonly known as co-stimulation, is previous activaon of the APC (Liu & Janeway Jr 1991; Banchereau &
einman 1998).
Based mostly on studies of autoreactive T cells, inter-
tion between CD40 ligand expressed by the T cell and

Steinman 1998).
Based mostly on studies of autoreactive T cells, inter-
action between CD40 ligand expressed by the T cell and
CD40 expressed by the APC has been found to be critical Based mostly on studies of autoreactive T cells, inter-
action between CD40 ligand expressed by the T cell and
CD40 expressed by the APC has been found to be critical
to this process (Grewal *et al.* 1996; Grewal & Elavel action between CD40 ligand expressed by the Tcell and CD40 expressed by the APC has been found to be critical
to this process (Grewal *et al.* 1996; Grewal & Flavell
1996–1998) Initial studies in virus-infected animals CD40 expressed by the APC has been found to be critical
to this process (Grewal *et al.* 1996; Grewal & Flavell
1996, 1998). Initial studies in virus-infected animals
quickly revealed that viral infection might by-pass th to this process (Grewal *et al.* 1996; Grewal & Flavell 1996, 1998). Initial studies in virus-infected animals quickly revealed that viral infection might by-pass the 1996, 1998). Initial studies in virus-infected animals
quickly revealed that viral infection might by-pass the
need for CD40-CD40 ligand in the activation of the
 CDR^+ T-cell response (Borrow et al. 1996; Whitnire et al. quickly revealed that viral infection might by-pass the need for CD40-CD40 ligand in the activation of the CD8⁺ T-cell response (Borrow *et al.* 1996; Whitmire *et al.* 1996). Complementing these findings it has been sho CD8⁺ T-cell response (Borrow *et al.* 1996; Whitmire *et al.* 1996). Complementing these findings it has been shown *in vitro* that viral infection of APCs may have the same effect on these cells as ligation of CD40 (Wu & Liu 1994). *vitro* that viral infection of APCs may have the same effect
on these cells as ligation of CD40 (Wu & Liu 1994).
However, as it was the case in $CD4^+$ T-cell-deficient mice,
subsequent extended analysis revealed a more c on these cells as ligation of CD40 (Wu & Liu 1994).
However, as it was the case in CD4⁺ T-cell-deficient mice,
subsequent extended analysis revealed a more complex
picture. Thus, while CD40 ligand expression was dis-However, as it was the case in CD4⁺ T-cell-deficient mice,
subsequent extended analysis revealed a more complex
picture. Thus, while CD40 ligand expression was dis-
pensable during the acute response to slowly replicatin subsequent extended analysis revealed a more complex
picture. Thus, while CD40 ligand expression was dis-
pensable during the acute response to slowly replicating picture. Thus, while CD40 ligand expression was dis-
pensable during the acute response to slowly replicating
LCMVArmstrong (Whitmire *et al.* 1996, 1999; Thomsen *et*
al. 1998b: Borrow *et al.* 1996), the primary response pensable during the acute response to slowly replicating LCMV Armstrong (Whitmire *et al.* 1996, 1999; Thomsen *et al.* 1998*b*; Borrow *et al.* 1996), the primary response collarsed rapidly in LCMV Traub-infected CD40 lig LCMV Armstrong (Whitmire *et al.* 1996, 1999; Thomsen *et al.* 1998*b*; Borrow *et al.* 1996), the primary response collapsed rapidly in LCMV Traub-infected CD40 ligand-
deficient mice (Thomsen *et al.* 1998*b*). In the l al. 1998*b*; Borrow *et al.* 1996), the primary response collapsed rapidly in LCMV Traub-infected CD40 ligand-
deficient mice (Thomsen *et al.* 1998*b*). In the latter mice,

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initial $CD8^+$ T-cell proliferation and differentiation was
normal, but already around day 10 p i the fraction of initial CD8⁺ T-cell proliferation and differentiation was
normal, but already around day 10 p.i., the fraction of
activated CD8⁺ T cells was rapidly shripking, and after initial CD8⁺ T-cell proliferation and differentiation was
normal, but already around day 10 p.i., the fraction of
activated CD8⁺ T cells was rapidly shrinking, and after
two months of infection no LCMV-specific memory normal, but already around day 10 p.i., the fraction of
activated CD8⁺ T cells was rapidly shrinking, and after
two months of infection no LCMV-specific memory cells
could be detected. Subsequent studies involving other activated CD8⁺ T cells was rapidly shrinking, and after
two months of infection no LCMV-specific memory cells
could be detected. Subsequent studies involving other
visceratronic strains have confirmed this nicture (A R two months of infection no LCMV-specific memory cells
could be detected. Subsequent studies involving other
viscerotropic strains have confirmed this picture (A. R. could be detected. Subsequent studies involving other viscerotropic strains have confirmed this picture (A. R. Thomsen, unpublished observation; Whitmire *et al.* 1999). With regard to virus control a matching pattern was scerotropic strains have confirmed this picture (A, R, A)
nomsen, unpublished observation; Whitmire *et al.* 1999).
With regard to virus control, a matching pattern was
served: some mice were able to transiently control th

Thomsen, unpublished observation; Whitmire *et al.* 1999).
With regard to virus control, a matching pattern was
observed: some mice were able to transiently control the
infection but after two months all were viraemic and harboured high titres of virus in their organs. In LCMV infection, but after two months all were viraemic and
harboured high titres of virus in their organs. In LCMV
Armstrong-infected mice, on the other hand, the infection
was initially controlled very efficiently i.e. below t harboured high titres of virus in their organs. In LCMV
Armstrong-infected mice, on the other hand, the infection
was initially controlled very efficiently, i.e. below the level
of detection, but, after about four to five was initially controlled very efficiently, i.e. below the level
of detection, but after about four to five months virus was initially controlled very efficiently, i.e. below the level
of detection, but after about four to five months virus
reappeared in the blood and substantial amounts were
generally detected in the organs (Thomsen *et al* of detection, but after about four to five months virus
reappeared in the blood and substantial amounts were
generally detected in the organs (Thomsen *et al.* 1998*b*).
Extended analysis of the LCMV-specific CD8⁺ T cell generally detected in the organs (Thomsen *et al.* 1998*b*).
Extended analysis of the LCMV-specific CDB^+ T cells in
LCMV Armstrong-infected CD40 ligand-deficient mice
(Andreasen *et al.* 2000) revealed that although ini Extended analysis of the LCMV-specific CD8⁺ T cells in
LCMV Armstrong-infected CD40 ligand-deficient mice
(Andreasen *et al.* 2000) revealed that although initially
canable of responding normally the per-cell canacity to LCMV Armstrong-infected CD40 ligand-deficient mice
(Andreasen *et al.* 2000) revealed that although initially
capable of responding normally, the per-cell capacity to
produce IFN- γ was significantly reduced with time. (Andreasen *et al.* 2000) revealed that although initially capable of responding normally, the per-cell capacity to produce IFN- γ was significantly reduced with time. Thus at two and four months n i mean fluorescence w capable of responding normally, the per-cell capacity to
produce IFN- γ was significantly reduced with time. Thus
at two and four months p.i., mean fluorescence was
markedly reduced and pentide-stimulated release of produce IFN- γ was significantly reduced with time. Thus
at two and four months p.i., mean fluorescence was
markedly reduced, and peptide-stimulated release of
IFN- γ was reduced by a factor of ten in CD40 ligandat two and four months p.i., mean fluorescence was
markedly reduced, and peptide-stimulated release of
IFN-γ was reduced by a factor of ten in CD40 ligand-
deficient mice compared to matched wild-type mice markedly reduced, and peptide-stimulated release of IFN- γ was reduced by a factor of ten in CD40 ligand-
deficient mice compared to matched wild-type mice. IFN- γ was reduced by a factor of ten in CD40 ligand-
deficient mice compared to matched wild-type mice.
Thus, even in LCMV Armstrong-infected mice in which
the virus load is virtually undetectable for a period of at deficient mice compared to matched wild-type mice.
Thus, even in LCMV Armstrong-infected mice in which
the virus load is virtually undetectable for a period of at
least two months, virus-specific CD8⁺ T cells appear to Thus, even in LCMV Armstrong-infected mice in which
the virus load is virtually undetectable for a period of at
least two months, virus-specific $CD8^+$ T cells appear to
be driven towards a state of apergy in the absence the virus load is virtually undetectable for a period of at
least two months, virus-specific CD8⁺ T cells appear to
be driven towards a state of anergy in the absence of
CD40 ligand expression. The result of this is reap least two months, virus-specific CD8⁺ T cells appear to
be driven towards a state of anergy in the absence of
CD40 ligand expression. The result of this is reappear-
ance of detectable levels of virus be driven towards a state of and
CD40 ligand expression. The rese
ance of detectable levels of virus.
In conclusion, these findings

ance of detectable levels of virus.

In conclusion, these findings reveal that although

CD40–CD40 ligand interaction is dispensable during the

acute response to LCMV the maintenance of an efficient In conclusion, these findings reveal that although
CD40–CD40 ligand interaction is dispensable during the
acute response to LCMV, the maintenance of an efficient
CD8⁺ T-cell population somehow requires this molecular CD40-CD40 ligand interaction is dispensable during the
acute response to LCMV, the maintenance of an efficient
CD8⁺ T-cell population somehow requires this molecular
interaction even under conditions where the virus load acute response to LCMV, the maintenance of an efficient CD8⁺ T-cell population somehow requires this molecular interaction even under conditions where the virus load is CD8⁺ T-cell population somehow requires this molecular
interaction even under conditions where the virus load is
limited. However, following infection with rapidly repli-
cating variants the consequences of this subontim interaction even under conditions where the virus load is
limited. However, following infection with rapidly repli-
cating variants, the consequences of this suboptimal
stimulation are aggravated and the result is a total limited. However, following infection with rapidly replicating variants, the consequences of this suboptimal
stimulation are aggravated, and the result is a total
collapse of the CD8⁺ T-cell response cating variants, the consequences of this suboptimal stimulation are aggravated, and the result is a total collapse of the $CDB⁺ T$ -cell response.

At the present time it is not completely clear whether collapse of the CD8⁺ T-cell response.
At the present time it is not completely clear whether
CD40 ligand needs to be expressed by the CD8⁺ T cells
themselves or by the CD4⁺ helper cells. However a At the present time it is not completely clear whether
CD40 ligand needs to be expressed by the CDB^+ T cells
themselves or by the $CD4^+$ helper cells. However, a
recent model for CD4 help states that $CD4^+$ T cells CD40 ligand needs to be expressed by the $CD8^+$ T cells
themselves or by the $CD4^+$ helper cells. However, a
recent model for $CD4$ help states that $CD4^+$ T cells
provide help primarily through 'conditioning' of the themselves or by the $CD4^+$ helper cells. However, a
recent model for $CD4$ help states that $CD4^+$ T cells
provide help primarily through 'conditioning' of the
APCs and that this requires expression of $CD40$ ligand recent model for CD4 help states that $CD4^+$ T cells
provide help primarily through 'conditioning' of the
APCs, and that this requires expression of CD40 ligand
(Bennett *et al.* 1998: Ridge *et al.* 1998: Schoenberger *e* provide help primarily through 'conditioning' of the APCs, and that this requires expression of CD40 ligand (Bennett *et al.* 1998; Ridge *et al.* 1998; Schoenberger *et al.* 1998) A likely scenario is therefore, that whil APCs, and that this requires expression of CD40 ligand (Bennett *et al.* 1998; Ridge *et al.* 1998; Schoenberger *et al.* 1998). A likely scenario is therefore, that while virally (Bennett *et al.* 1998; Ridge *et al.* 1998; Schoenberger *et al.* 1998). A likely scenario is therefore, that while virally induced activation of the APCs suffices during the early phase of infection (Andreasen *et al.* 1998). A likely scenario is therefore, that while virally induced activation of the APCs suffices during the early phase of infection (Andreasen *et al.* 2000)—driving the initial CDB^+ T-cell expansion and differentiati phase of infection (Andreasen *et al.* 2000)—driving the initial $CD8^+$ T-cell expansion and differentiation—this phase of infection (Andreasen *et al.* 2000)—driving the initial $CD8^+$ T-cell expansion and differentiation—this signal wanes with time, and $CD40$ ligand-mediated acti-
vation therefore becomes a limiting factor in maint initial CD8⁺ T-cell expansion and differentiation—this
signal wanes with time, and CD40 ligand-mediated acti-
vation therefore becomes a limiting factor in maintaining
optimally stimulating APCs required for the maintena signal wanes with time, and CD40 ligand-mediated activation therefore becomes a limiting factor in maintaining
optimally stimulating APCs required for the maintenance
of effector activity in the CD8⁺ population. This int vation therefore becomes a limiting factor in maintaining
optimally stimulating APCs required for the maintenance
of effector activity in the CD8⁺ population. This inter-
pretation is also consistent with the findings pr proposimally stimulating APCs required for the maintenance
of effector activity in the CDB^+ population. This inter-
pretation is also consistent with the findings previously
made in $CD4^+$ T-cell-deficient mice (Thomsen of effector activity in the CDB^+ population. This inter-
pretation is also consistent with the findings previously
made in $CD4^+$ T-cell-deficient mice (Thomsen *et al.*
1996) and thus constitutes a simple consensus mod pretation is also consistent with the findings previously
made in $CD4^+$ T-cell-deficient mice (Thomsen *et al.* 1996), and thus constitutes a simple consensus model for made in $CD4^+$ T-cell-deficient mice (Thomsen *et al.* 1996), and thus constitutes a simple consensus model for maintenance of $CD8^+$ T-cell function in persistently infected animals 1996), and thus co
maintenance of C
infected animals. $\begin{array}{ll}\text{in the case of } & \text{CD8}^+ & \text{T-cell function in } \text{persistently} \ \text{fected animals.} \end{array}$
Given that a requirement for ongoing interaction with PCs is intrinsic to the above model how can this be

infected animals.
Given that a requirement for ongoing interaction with
APCs is intrinsic to the above model, how can this be reconciled with recent findings indicating that memory

With regard to virus control, a matching pattern was
observed: some mice were able to transiently control the
infection, but after two months all were viraemic and
harboured high titres of virus in their organs. In LCMV T generally detected in the organs (Thomsen *et al.* 1998b). specificities. If indeed these results were applied without Extended analysis of the LCMV-specific $CD8^+$ T cells in modification to the situation in a normal hos CD40 ligand expression. The result of this is reappear-of a persistent infection that requires long-standing T-cell
ance of detectable levels of virus.
In conclusion, these findings reveal that although context, it might b CD8⁺ T cells are maintained and may even proliferate in
the absence of antigenic stimulation and cellular CD8⁺ T cells are maintained and may even proliferate in
the absence of antigenic stimulation and cellular
interactions (Di Rosa & Matzinger 1996; Hou *et al* CD8⁺ Tcells are maintained and may even proliferate in
the absence of antigenic stimulation and cellular
interactions (Di Rosa & Matzinger 1996; Hou *et al.*
1994: Lau *et al.* 1994: Murali-Krishna *et al.* 1999)? We the absence of antigenic stimulation and cellular interactions (Di Rosa & Matzinger 1996; Hou *et al.* 1994; Lau *et al.* 1994; Murali-Krishna *et al.* 1999)? We believe that the answer to this question lies in the experiinteractions (Di Rosa & Matzinger 1996; Hou *et al.* 1994; Lau *et al.* 1994; Murali-Krishna *et al.* 1999)? We believe that the answer to this question lies in the experimental conditions under which the latter findings 1994; Lau *et al.* 1994; Murali-Krishna *et al.* 1999)? We believe that the answer to this question lies in the experimental conditions under which the latter findings have believe that the answer to this question lies in the experimental conditions under which the latter findings have
been obtained. Thus, we find it pertinent that the
evidence supporting presentation-independent maintemental conditions under which the latter findings have
been obtained. Thus, we find it pertinent that the
evidence supporting presentation-independent mainte-
nance of memory $CD8^+$ T cells mostly derives from been obtained. Thus, we find it pertinent that the evidence supporting presentation-independent maintenance of memory CDB^+ T cells mostly derives from experiments involving adoptive transfer of primed CDB^+ nance of memory $CD8^+$ T cells mostly derives from experiments involving adoptive transfer of primed CD8⁺
Tcells into a MHC class I-free environment. This constitutes a highly contrived situation in which the memory
CD8⁺ Tcells exist in an immunological vacuum removed T cells into a MHC class I-free environment. This constitutes a highly contrived situation in which the memory CD8⁺ T cells exist in an immunological vacuum removed from interactions with the surrounding environment as $CD8⁺$ T cells exist in an immunological vacuum removed from interactions with the surrounding environment as $CDB⁺ T cells exist in an immunological vacuum removed from interactions with the surrounding environment as well as from competition from expanding clones of other specificities. If indeed these results were anplied without$ from interactions with the surrounding environment as
well as from competition from expanding clones of other
specificities. If indeed these results were applied without
modification to the situation in a normal host the c well as from competition from expanding clones of other
specificities. If indeed these results were applied without
modification to the situation in a normal host, the conse-
quence would be that once generated a memory su specificities. If indeed these results were applied without modification to the situation in a normal host, the consequence would be that once generated, a memory subset would persist forever. Given that there is a natural quence would be that once generated, a memory subset
would persist forever. Given that there is a natural upper
limit to the total number of T cells (and probably to the quence would be that once generated, a memory subset
would persist forever. Given that there is a natural upper
limit to the total number of T cells (and probably to the
number of memory cells *her* se: Tanchot & Rocha 199 would persist forever. Given that there is a natural upper
limit to the total number of T cells (and probably to the
number of memory cells *per se*; Tanchot & Rocha 1998),
this would—in the perspective of a lifetime—leave limit to the total number of T cells (and probably to the number of memory cells *per* se ; Tanchot & Rocha 1998), this would—in the perspective of a lifetime—leave very little room for dynamic adaptation of the T-cell re number of memory cells *per se*; Tanchot & Rocha 1998), this would—in the perspective of a lifetime—leave very little room for dynamic adaptation of the T-cell repertoire in the face of a changing environment. Consequentl this would—in the perspective of a lifetime—leave very
little room for dynamic adaptation of the T-cell repertoire
in the face of a changing environment. Consequently,
although the available evidence indicates that under little room for dynamic adaptation of the T-cell repertoire
in the face of a changing environment. Consequently,
although the available evidence indicates that, under in the face of a changing environment. Consequently,
although the available evidence indicates that, under
certain conditions, a memory $CD8^+$ T-cell population
may be maintained in viva in the absence of T-cell although the available evidence indicates that, under
certain conditions, a memory CDB^+ T-cell population
may be maintained *in vivo* in the absence of T-cell
receptor stimulation we would tend to believe that this is certain conditions, a memory $CD8^+$ T-cell population
may be maintained *in vivo* in the absence of T-cell
receptor stimulation, we would tend to believe that this is
not generally the situation. And particularly in the c may be maintained *in vivo* in the absence of T-cell
receptor stimulation, we would tend to believe that this is
not generally the situation. And particularly in the case
of a persistent infection that requires long-stand receptor stimulation, we would tend to believe that this is
not generally the situation. And particularly in the case
of a persistent infection that requires long-standing T-cell
surveillance different rules are likely to not generally the situation. And particularly in the case
of a persistent infection that requires long-standing T-cell
surveillance, different rules are likely to apply. In this
context, it might be relevant to bear in min of a persistent infection that requires long-standing T-cell
surveillance, different rules are likely to apply. In this
context, it might be relevant to bear in mind that
memory probably constitutes an evolutionary design surveillance, different rules are likely to apply. In this context, it might be relevant to bear in mind that memory probably constitutes an evolutionary design for the control of persistent infections rather than a context, it might be relevant to bear in mind that
memory probably constitutes an evolutionary design for
the control of persistent infections rather than a
mechanism for protection against exogenous rechallenge memory probably constitutes an evolutionary design for
the control of persistent infections rather than a
mechanism for protection against exogenous rechallenge
(Wodarz et al. 2000). Moreover, some of the findings the control of persistent infections rather than a
mechanism for protection against exogenous rechallenge
(Wodarz *et al.* 2000). Moreover, some of the findings
presented below provide further evidence for a dynamic mechanism for protection against exogenous rechallenge (Wodarz *et al.* 2000). Moreover, some of the findings presented below provide further evidence for a dynamic interaction continuing into the 'memory' phase of this (Wodarz *et al.* 2000). Moreover, some of the findings presented below provide further evidence for a dynamic interaction continuing into the 'memory' phase of this persistent infection presented below provinteraction continuin
persistent infection.

6. EFFECTOR MOLECULES INVOLVED IN THE CONTROL OF INFECTION WITH A NON-CYTOCIDAL VIRUS

VIRUS
VIRUS
Although CD8⁺ Tcells are often called by their eponym
FI_s and are assumed by many to serve predominantly Although CD8⁺ Tcells are often called by their eponym
CTLs, and are assumed by many to serve predominantly
as effectors in cell contact-dependent killing, they are also Although $CD8^+$ Tcells are often called by their eponym CTLs, and are assumed by many to serve predominantly as effectors in cell contact-dependent killing, they are also potent producers of several cytokines and may like CTLs, and are assumed by many to serve predominantly
as effectors in cell contact-dependent killing, they are also
potent producers of several cytokines and may, like CD4⁺
cells, be divided into at least two major functi as effectors in cell contact-dependent killing, they are also
potent producers of several cytokines and may, like $CD4^+$
cells, be divided into at least two major functional subsets
based on their cytokine profiles (Carte potent producers of several cytokines and may, like CD4⁺ cells, be divided into at least two major functional subsets based on their cytokine profiles (Carter & Dutton 1996). Tc1 cells produce essentially the same cytokines as Th1 based on their cytokine profiles (Carter & Dutton 1996).
Tell cells produce essentially the same cytokines as Thi
cells, in particular IFN- γ , the detection of which may be
used as a means to quantitate $CD8^+$ responses Tel cells produce essentially the same cytokines as Thi
cells, in particular IFN- γ , the detection of which may be
used as a means to quantitate $CD8^+$ responses (see § 4). Tc2
cells have the same cytokine profile as Th cells, in particular IFN- γ , the detection of which may be
used as a means to quantitate $CD8^+$ responses (see § 4). Tc2
cells have the same cytokine profile as Th2 cells and may
be detected by demonstration of H₋₅ in used as a means to quantitate $CD8^+$ responses (see § 4). Tc2
cells have the same cytokine profile as Th2 cells and may
be detected by demonstration of IL-5 intracellularly. In cells have the same cytokine profile as Th2 cells and may
be detected by demonstration of IL-5 intracellularly. In
general, viral infections are associated with Tcl responses,
whereas Tc2 cells are only found rarely (Sede be detected by demonstration of IL-5 intracellularly. In general, viral infections are associated with Tcl responses, whereas Tc2 cells are only found rarely (Seder & Le Gros 1995: Christensen *et al.* 1996) general, viral infections are as:
whereas Tc2 cells are only four
1995; Christensen *et al.* 1996).
In itself, the generality of the nereas Tc2 cells are only found rarely (Seder & Le Gros
95; Christensen *et al.* 1996).
In itself, the generality of this pattern could be taken to
greet, that the production of type 1 cytokines would

1995; Christensen *et al.* 1996).
In itself, the generality of this pattern could be taken to suggest that the production of type 1 cytokines would
serve some functional purpose during the resolution of In itself, the generality of this pattern could be taken to suggest that the production of type 1 cytokines would serve some functional purpose during the resolution of most viral infections. However, in the case of nonsuggest that the production of type 1 cytokines would
serve some functional purpose during the resolution of
most viral infections. However, in the case of non-
cytocidal viruses it has been suggested that production of serve some functional purpose during the resolution of

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 $IFN-\gamma$ is virtually redundant, whereas cell-dependent
killing primarily via the perform pathway is essential to IFN- γ is virtually redundant, whereas cell-dependent
killing, primarily via the perforin pathway, is essential to
virus elimination (Kagi & Hengartner 1996) However IFN- γ is virtually redundant, whereas cell-dependent
killing, primarily via the perforin pathway, is essential to
virus elimination (Kagi & Hengartner 1996). However,
this suggestion is based mostly on the study of LCM killing, primarily via the perforin pathway, is essential to
virus elimination (Kagi & Hengartner 1996). However,
this suggestion is based mostly on the study of LCMV
Armstrong-infected mice analysed during the acute phase virus elimination (Kagi & Hengartner 1996). However,
this suggestion is based mostly on the study of LCMV
Armstrong-infected mice analysed during the acute phase
of infection, and as the following will reveal, this reprethis suggestion is based mostly on the study of LCMV
Armstrong-infected mice analysed during the acute phase
of infection, and as the following will reveal, this repre-Armstrong-infected mice analysed during the acute phase
of infection, and as the following will reveal, this repre-
sents a far from complete picture as regards the involve-
ment of IFN-v in control of the LCMV infection ment of IFN- γ in control of the LCMV infection.
The first indication that IFN- γ might play a role in the a far from complete picture as regards the involvent of IFN-γ in control of the LCMV infection.
The first indication that IFN-γ might play a role in trolling this infection came from findings in virus

controlling this infection came from findings in virus carrier mice adoptively immunized with primed donor controlling this infection came from findings in virus
carrier mice adoptively immunized with primed donor
cells. In this situation, capacity of the donor cells to
produce IFN-v was found to be of critical importance carrier mice adoptively immunized with primed donor
cells. In this situation, capacity of the donor cells to
produce IFN- γ was found to be of critical importance
(Tishon *et al* 1995; Planz *et al* 1997) This finding l cells. In this situation, capacity of the donor cells to produce IFN- γ was found to be of critical importance (Tishon *et al.* 1995; Planz *et al.* 1997). This finding led us to pursue the role of IFN- γ in the course produce IFN- γ was found to be of critical importance (Tishon *et al.* 1995; Planz *et al.* 1997). This finding led us to pursue the role of IFN- γ in the course of a normal LCMV infection in adult mice Based on the a (Tishon *et al.* 1995; Planz *et al.* 1997). This finding led us to pursue the role of IFN- γ in the course of a normal LCMV infection in adult mice. Based on the assumption that cytokines were likely to be most importa pursue the role of IFN- γ in the course of a normal
LCMV infection in adult mice. Based on the assumption
that cytokines were likely to be most important during
infection with a rapidly replicating virus strain IFN- γ LCMV infection in adult mice. Based on the assumption
that cytokines were likely to be most important during
infection with a rapidly replicating virus strain, IFN- γ -
deficient mice and wild-type controls were infected infection with a rapidly replicating virus strain, IFN- γ -
deficient mice and wild-type controls were infected with
200 pfu of LCMV Traub. Using these conditions (Nansen
et al. 1999) we found that organ virus titres in deficient mice and wild-type controls were infected with
200 pfu of LCMV Traub. Using these conditions (Nansen
et al. 1999) we found that organ virus titres in IFN- γ -
deficient mice on day 10 p.i. were as high as in m *et al.* 1999) we found that organ virus titres in IFN- γ -
deficient mice on day 10 p.i. were as high as in mice
having no T cells at all. With time, virus levels became
somewhat reduced but the maiority of infected IFN deficient mice on day 10 p.i. were as high as in mice sug
having no T cells at all. With time, virus levels became the
somewhat reduced, but the majority of infected IFN- γ - virus
deficient mice succumbed in the process somewhat reduced, but the majority of infected IFN- γ -
deficient mice succumbed in the process $\langle a, 85\% \rangle$
mortality). This outcome of infection was due to CD8⁺ T-
cell-dependent immunopathology since depletion of t deficient mice succumbed in the process $\langle a. 85\% \rangle$ mortality). This outcome of infection was due to $CD8^+$ T-
cell-dependent immunopathology, since depletion of this
subset protected the mice With the use of newly gene mortality). This outcome of infection was due to CD8⁺ T-
cell-dependent immunopathology, since depletion of this
subset protected the mice. With the use of newly gener-
ated perforin–IFN- χ double knockout mice, we cou cell-dependent immunopathology, since depletion of this deficient mice infected with LCMV Armstrong supports subset protected the mice. With the use of newly gener- our assumption that persisting antigen may play an ated p further demonstrate that perforin-mediated cell killing ated perforin–IFN- γ double knockout mice, we could
further demonstrate that perforin-mediated cell killing
was a central, though not the only, effector mechanism.
Although most double knockout mice survived (ca. 90%) further demonstrate that perforin-mediated cell killing
was a central, though not the only, effector mechanism.
Although most double knockout mice survived (*ca*. 90%),
transient CD⁸⁺ T-cell-mediated wasting was noted Ve was a central, though not the only, effector mechanism.
Although most double knockout mice survived $(a. 90\%)$,
transient $CD8^+$ T-cell-mediated wasting was noted. Very
limited disease was observed in similarly infected wi Although most double knockout mice survived $(ca. 90\%)$,
transient $CD8^+$ T-cell-mediated wasting was noted. Very
limited disease was observed in similarly infected wildtransient CD8⁺ T-cell-mediated wasting was noted. Very
limited disease was observed in similarly infected wild-
type mice. Thus, the inability to produce IFN- γ resulted
in augmented virus levels in the viscera, and un limited disease was observed in similarly infected wild-
type mice. Thus, the inability to produce IFN- γ resulted
in augmented virus levels in the viscera, and under these
conditions CTI killing of infected cells led t type mice. Thus, the inability to produce $IFN-\gamma$ resulted
in augmented virus levels in the viscera, and under these
conditions CTL killing of infected cells led to progressive
tissue destruction and death in augmented virus levels in the
conditions CTL killing of infer
tissue destruction and death.
In contrast IFN-v played nditions CTL killing of infected cells led to progressive
sue destruction and death.
In contrast, IFN-γ played a relatively minor role in
NMV Armstrong-infected mice and all survived the

tissue destruction and death.
In contrast, IFN- γ played a relatively minor role in LCMV Armstrong-infected mice, and all survived the In contrast, IFN- γ played a relatively minor role in LCMV Armstrong-infected mice, and all survived the acute phase. Thus, as opposed to perforin, which was essential for virus control irrespective of replication rate LCMV Armstrong-infected mice, and all survived the acute phase. Thus, as opposed to perforin, which was essential for virus control irrespective of replication rate, the importance of $IFN-x$ varied with this viral paramete acute phase. Thus, as opposed to perforin, which was
essential for virus control irrespective of replication rate,
the importance of IFN- γ varied with this viral parameter
(Nansen *et al.* 1999). However, a small influ essential for virus control irrespective of replication rate, the importance of IFN- γ varied with this viral parameter (Nansen *et al.* 1999). However, a small influence was the importance of IFN- γ varied with this viral parameter
(Nansen *et al.* 1999). However, a small influence was
noted, and extended analysis of virus levels in LCMV
Armstrong-infected IFN- γ -deficient mice revealed t (Nansen *et al.* 1999). However, a small influence was noted, and extended analysis of virus levels in LCMV Armstrong-infected IFN- γ -deficient mice revealed that the infection was never completely controlled and signif noted, and extended analysis of virus levels in LCMV
Armstrong-infected IFN- γ -deficient mice revealed that
the infection was never completely controlled and signifi-
cant levels of virus could be demonstrated in spleen Armstrong-infected IFN- γ -deficient mice revealed that
the infection was never completely controlled and signifi-
cant levels of virus could be demonstrated in spleen and
lungs for months after infection (Bartholdy *et* the infection was never completely controlled and signifi-Notably, no impairment of either effector or memory Tlungs for months after infection (Bartholdy *et al.* 2000).
Notably, no impairment of either effector or memory T-
cell generation was observed in these mice as evidenced
by supranormal *ex nine* CTI activity and increase Notably, no impairment of either effector or memory T-
cell generation was observed in these mice as evidenced
by supranormal *ex vivo* CTL activity and increased
numbers of CTL precursors as determined by limiting cell generation was observed in these mice as evidenced
by supranormal ex vivo CTL activity and increased
numbers of CTL precursors as determined by limiting
dilution. In fact, ex vivo CTL activity remained subby supranormal *ex vivo* CTL activity and increased numbers of CTL precursors as determined by limiting dilution. In fact, *ex vivo* CTL activity remained subnumbers of CTL precursors as determined by limiting
dilution. In fact, ex vivo CTL activity remained sub-
stantial for many months in chronically infected IFN- γ -
deficient mice, whereas this response gradually
disappea or stantial for many months in chronically infected IFN-γ-
deficient mice, whereas this response gradually
disappeared in wild-type mice. Also in this situation,
analysis of cell-cycle and phenotypic markers provided deficient mice, whereas this response gradually al. 1998). In the context of the studies mentioned above, disappeared in wild-type mice. Also in this situation, we consistently noted that the choice of viral strain was ana disappeared in wild-type mice. Also in this situation,
analysis of cell-cycle and phenotypic markers provided
additional support for chronic ongoing CD8⁺ T-cell
activation in persistently infected mice. Thus, it appears analysis of cell-cycle and phenotypic markers provided
additional support for chronic ongoing CDB^+ T-cell
activation in persistently infected mice. Thus, it appears
that a new equilibrium is established in $LCMV$ additional support for chronic ongoing CD8⁺ T-cell
activation in persistently infected mice. Thus, it appears
that a new equilibrium is established in LCMV A
Armstrong-infected IFN-x-deficient mice with long-term activation in persistently infected mice. Thus, it appears mice were infected with a rapidly invasive strain.

that a new equilibrium is established in LCMV Although this may intuitively be felt as an obvious

Armstrong-i coexistence of cytolytically active $CD8⁺$ effector cells and

target cells in several organs supporting active replication
of the virus target cells in
of the virus.
In conclusie

In conclusion, our results demonstrate that IFN-^g is by of the virus.
In conclusion, our results demonstrate that IFN- γ is by
no means redundant to the control of LCMV infection.
Indeed it is evident that failure to produce this cytokine In conclusion, our results demonstrate that IFN- γ is by
no means redundant to the control of LCMV infection.
Indeed, it is evident that failure to produce this cytokine
markedly affects the outcome of infection with th no means redundant to the control of LCMV infection.
Indeed, it is evident that failure to produce this cytokine
markedly affects the outcome of infection with this non-
cytocidal virus. Thus lack of IFN- γ may transform Indeed, it is evident that failure to produce this cytokine
markedly affects the outcome of infection with this non-
cytocidal virus. Thus lack of IFN- γ may transform an
essentially asymptomatic infection with a rapidl markedly affects the outcome of infection with this non-
cytocidal virus. Thus lack of $IFN-\gamma$ may transform an
essentially asymptomatic infection with a rapidly repli-
cating LCMV strain into a fatal disease, and infectio cytocidal virus. Thus lack of IFN- γ may transform an
essentially asymptomatic infection with a rapidly repli-
cating LCMV strain into a fatal disease, and infection
with a slowly replicating strain results in chronic l essentially asymptomatic infection with a rapidly replicating LCMV strain into a fatal disease, and infection with a slowly replicating strain results in chronic lowcating LCMV strain into a fatal disease, and infection
with a slowly replicating strain results in chronic low-
grade infection; in the latter case, the lack of cytokine
production is partially compensated by permanently with a slowly replicating strain results in chronic low-
grade infection; in the latter case, the lack of cytokine
production is partially compensated by permanently
increased CTL activity. An important feature of the IFNgrade infection; in the latter case, the lack of cytokine
production is partially compensated by permanently
increased CTL activity. An important feature of the IFN-
 γ –I.CMV Armstrong model is that although chronic Tproduction is partially compensated by permanently
increased CTL activity. An important feature of the IFN-
 γ –LCMV Armstrong model is that although chronic T-
cell stimulation is observed we find no evidence pointing increased CTL activity. An important feature of the IFN- γ –LCMV Armstrong model is that although chronic T-
cell stimulation is observed, we find no evidence pointing
towards a collanse of immune surveillance: viral tit γ –LCMV Armstrong model is that although chronic T-
cell stimulation is observed, we find no evidence pointing
towards a collapse of immune surveillance: viral titres
remain stable for months. This observation therefore cell stimulation is observed, we find no evidence pointing
towards a collapse of immune surveillance: viral titres
remain stable for months. This observation therefore high-
lights the fact that while chronic stimulation i towards a collapse of immune surveillance: viral titres
remain stable for months. This observation therefore high-
lights the fact that while chronic stimulation is clearly a
precondition for T-cell exhaustion, the conditi remain stable for months. This observation therefore high-
lights the fact that while chronic stimulation is clearly a
precondition for T-cell exhaustion, the conditions
surrounding this stimulation are essential in determ lights the fact that while chronic stimulation is clearly a
precondition for T-cell exhaustion, the conditions
surrounding this stimulation are essential in determining
the final outcome Thus interestingly preliminary resu precondition for T-cell exhaustion, the conditions
surrounding this stimulation are essential in determining
the final outcome. Thus, interestingly, preliminary results
suggest that $CD4^+$ T-cell depletion in this situati surrounding this stimulation are essential in determining
the final outcome. Thus, interestingly, preliminary results
suggest that CD4⁺ T-cell depletion in this situation tips
the scale in favour of the virus, resulting the final outcome. Thus, interestingly, preliminary results
suggest that CD4⁺ T-cell depletion in this situation tips
the scale in favour of the virus, resulting in increased
virus levels and immunonathology suggest that CD4⁺ T-cell depletion
the scale in favour of the virus, re
virus levels and immunopathology.
In a broader perspective the fine In a scale in favour of the virus, resulting in increased
virus levels and immunopathology.
In a broader perspective, the finding of a chronically

rian dimmunopathology.

In a broader perspective, the finding of a chronically

elevated level of CD8⁺ T-cell surveillance in IFN-γ-

deficient mice infected with LCMV Armstrong supports

our assumption that persisting elevated level of CD8⁺ T-cell surveillance in IFN- γ -
deficient mice infected with LCMV Armstrong supports
our assumption that persisting antigen may play an
important role in maintaining effector T-cell activity into deficient mice infected with LCMV Armstrong supports what is normally perceived as the memory phase. important role in maintaining effector T-cell activity into
what is normally perceived as the memory phase.
Although it may be argued that this represents a
contrived situation additional observations strongly what is normally perceived as the memory phase.
Although it may be argued that this represents a
contrived situation, additional observations strongly
suggest that the conclusion is valid also in the normal Although it may be argued that this represents a contrived situation, additional observations strongly suggest that the conclusion is valid also in the normal host. Thus not unlike the pattern in LCMV Armstrong. contrived situation, additional observations strongly
suggest that the conclusion is valid also in the normal
host. Thus, not unlike the pattern in LCMV Armstrongsuggest that the conclusion is valid also in the normal
host. Thus, not unlike the pattern in LCMV Armstrong-
infected, IFN-γ-deficient mice we find a somewhat
protracted CTL response in wild-type mice infected with host. Thus, not unlike the pattern in LCMV Armstrong-
infected, IFN- γ -deficient mice we find a somewhat
protracted CTL response in wild-type mice infected with
the more persistent LCMV Traub strain (figure 4) Moreinfected, IFN-γ-deficient mice we find a somewhat
protracted CTL response in wild-type mice infected with
the more persistent LCMV Traub strain (figure 4). More-
over preliminary data on virus-specific CD8⁺ T cells protracted CTL response in wild-type mice infected with
the more persistent LCMV Traub strain (figure 4). More-
over, preliminary data on virus-specific CD8⁺ T cells
enumerated through detection of intracellular IFN-*x* the more persistent LCMV Traub strain (figure 4). More-
over, preliminary data on virus-specific CD8⁺ T cells
enumerated through detection of intracellular IFN-γ also
reveal a more protracted response in LCMV Traub rela over, preliminary data on virus-specific $CD8^+$ T cells
enumerated through detection of intracellular IFN- γ also
reveal a more protracted response in LCMV Traub rela-
tive to LCMV Armstrong-infected wild-type mice (A R enumerated through detection of intracellular IFN- γ also
reveal a more protracted response in LCMV Traub rela-
tive to LCMV Armstrong-infected wild-type mice (A. R.
Thomsen, unpublished observation) reveal a more protracted response in
tive to LCMV Armstrong-infected wi
Thomsen, unpublished observation). Thomsen, unpublished observation).
7. THE USE OF MATHEMATICAL MODELLING

7. THE USE OF MATHEMATICAL MODELLING
AS A TOOL TO UNDERSTAND T-CELL DYNAMICS
AN IN VIRAL INFECTIONS OF MATHEMATICAL MOD
O UNDERSTAND T-CELL I
IN VIRAL INFECTIONS

IN VIRAL INFECTIONS
It is a classical observation that the capacity of an IN VIRAL INFECTIONS

It is a classical observation that the capacity of an

LCMV strain to induce T-cell exhaustion when inocu-

lated into normal adult mice at high doses critically It is a classical observation that the capacity of an
LCMV strain to induce T-cell exhaustion when inocu-
lated into normal adult mice at high doses critically
denends on the ability of the virus strain to rapidly replilated into normal adult mice at high doses critically depends on the ability of the virus strain to rapidly replilated into normal adult mice at high doses critically
depends on the ability of the virus strain to rapidly repli-
cate in the host. Although still primarily an empirical
observation some new insight into this phenomenon a depends on the ability of the virus strain to rapidly replicate in the host. Although still primarily an empirical observation, some new insight into this phenomenon and the underlying mechanisms has recently been gained b cate in the host. Although still primarily an empirical observation, some new insight into this phenomenon and the underlying mechanisms has recently been gained by application of simple mathematical simulation (Wodarz et observation, some new insight into this phenomenon and
the underlying mechanisms has recently been gained by
application of simple mathematical simulation (Wodarz et
 al , 1998). In the context of the studies mentioned ab the underlying mechanisms has recently been gained by application of simple mathematical simulation (Wodarz *et al.* 1998). In the context of the studies mentioned above, we consistently noted that the choice of viral stra application of simple mathematical simulation (Wodarz *et al.* 1998). In the context of the studies mentioned above, we consistently noted that the choice of viral strain was critical to the results obtained and that gene we consistently noted that the choice of viral strain was effect observed would tend to become more exaggerated if critical to the results obtained, and that generally any
effect observed would tend to become more exaggerated if
mice were infected with a rapidly invasive strain.
Although this may intuitively be felt as an obvious effect observed would tend to become more exaggerated if
mice were infected with a rapidly invasive strain.
Although this may intuitively be felt as an obvious
finding it is nevertheless noteworthy that the rate of viral mice were infected with a rapidly invasive strain.
Although this may intuitively be felt as an obvious
finding, it is nevertheless noteworthy that the rate of viral
replication may still continue to play a decisive role ev Although this may intuitively be felt as an obvious

Figure 4. Prolonged CTL activity in LCMV Armstong-
infected IFN-γ-deficient mice. IFN-γ-deficient and wild-
type were infected with 4800 plaque-forming units of LC infected IFN- γ -deficient mice. IFN- γ -deficient and wild-
type were infected with 4800 plaque-forming units of LCMV
Armstrong, for comparison an additional group of wild-type type were infected with 4800 plaque-forming units of LCMV type were infected with 4800 plaque-forming units of LCMV
Armstrong, for comparison an additional group of wild-type
mice were infected with 200 plaque-forming units of LCMV
Traub, Four weeks after infection, splenocytes w Armstrong, for comparison an additional group of wild-type
mice were infected with 200 plaque-forming units of LCMV
Traub. Four weeks after infection, splenocytes were harvested
and tested in a ⁵¹Cr-release assay against mice were infected with 200 plaque-forming units of LCMV
Traub. Four weeks after infection, splenocytes were harvested
and tested in a ⁵¹Cr-release assay against GP33-41 pulsed
RMA-S tumour cells (closed symbols): unpuls Traub. Four weeks after infection, splenocytes were harvested
and tested in a ⁵¹Cr-release assay against GP33-41 pulsed
RMA-S tumour cells (closed symbols); unpulsed RMA-S cells
served as control targets (open symbols). and tested in a ⁵¹Cr-release assay against GP33-41 pulsed
RMA-S tumour cells (closed symbols); unpulsed RMA-S cel
served as control targets (open symbols). Note that while
pentide-specific cytotoxicity in LCMV Armstrong-RMA-S tumour cells (closed symbols); unpulsed RMA-S cells
served as control targets (open symbols). Note that while
peptide-specific cytotoxicity in LCMV Armstrong-infected
wild-type mice has declined to a low level, subst served as control targets (open symbols). Note that while
peptide-specific cytotoxicity in LCMV Armstrong-infect
wild-type mice has declined to a low level, substantial
cytotoxic activity is still observed in similarly inf peptide-specific cytotoxicity in LCMV Armstrong-infecte
wild-type mice has declined to a low level, substantial
cytotoxic activity is still observed in similarly infected
IEN-x-deficient mice as well as in wild-type mice i wild-type mice has declined to a low level, substantial
cytotoxic activity is still observed in similarly infected
IFN-γ-deficient mice as well as in wild-type mice infected
with a lower dose of the more persistent LCMV Tr cytotoxic activity is still observed in similarly infected
IFN-γ-deficient mice as well as in wild-type mice infected
with a lower dose of the more persistent LCMV Traub.

with a lower dose of the more persistent LCMV Traub.
at a relatively late stage in a more or less chronic infecat a relatively late stage in a more or less chronic infection. A good example of this may be found in the observation that intravenous infection with a very low at a relatively late stage in a more or less chronic infection. A good example of this may be found in the ephotervation that intravenous infection with a very low before of the viscerotronic Trauh strain (2 pfu) resul tion. A good example of this may be found in the
observation that intravenous infection with a very low
dose of the viscerotropic Traub strain (2 pfu) results in a
more rapid and marked breakdown of virus control in observation that intravenous infection with a very low be obtained.
dose of the viscerotropic Traub strain (2 pfu) results in a Similar t
more rapid and marked breakdown of virus control in various conc dose of the viscerotropic Traub strain (2 pfu) results in a
more rapid and marked breakdown of virus control in
CD40 ligand-deficient mice than does infection of the
same mice with a 1000-fold higher dose of slowly replimore rapid and marked breakdown of virus control in
CD40 ligand-deficient mice than does infection of the
same mice with a 1000-fold higher dose of slowly repli-
cating LCMV Armstrong (Thomsen *et al.* 1998*b*). To CD40 ligand-deficient mice than does infection of the same mice with a 1000-fold higher dose of slowly replicating LCMV Armstrong (Thomsen *et al.* 1998*b*). To understand this better we modified the original mathemasame mice with a 1000-fold higher dose of slowly replicating LCMV Armstrong (Thomsen *et al.* 1998*b*). To understand this better we modified the original mathematical model describing the basic interactions between cating LCMV Armstrong (Thomsen *et al.* 1998*b*). To understand this better we modified the original mathematical model describing the basic interactions between virus replication and CTI response (Nowak & Bangham understand this better we modified the original mathematical model describing the basic interactions between of
virus replication and CTL response (Nowak & Bangham is
1996) so as to accommodate the above interpretation tical model describing the basic interactions between
virus replication and CTL response (Nowak & Bangham
1996) so as to accommodate the above interpretation
regarding the role of $CD4^+$ T cells–APCs in regulating virus replication and CTL response (Nowak & Bangham 1996) so as to accommodate the above interpretation regarding the role of $CD4^+$ T cells–APCs in regulating the dynamics of the CTL response (D Wodarz and A \overline{R}) 1996) so as to accommodate the above interpretation regarding the role of $CD4^+$ T cells-APCs in regulating the dynamics of the CTL response (D. Wodarz and A. R. Thomsen, unpublished observation) Without going into regarding the role of CD4⁺ T cells–APCs in regulating
the dynamics of the CTL response (D. Wodarz and A. R.
Thomsen, unpublished observation). Without going into
the details of our modifications—this analysis still the dynamics of the CTL response (D. Wodarz and A. R.
Thomsen, unpublished observation). Without going into
the details of our modifications—this analysis still
requires some additional evaluation—it may be stated Thomsen, unpublished observation). Without going into
the details of our modifications—this analysis still
requires some additional evaluation—it may be stated
that the predictions so far fit the experimental observathe details of our modifications—this analysis still
requires some additional evaluation—it may be stated
that the predictions so far fit the experimental observa-
tions well. For example simply by putting an upper limit requires some additional evaluation—it may be stated
that the predictions so far fit the experimental observa-
tions well. For example, simply by putting an upper limit
to the number of cell divisions CTLs can undergo in t that the predictions so far fit the experimental observations well. For example, simply by putting an upper limit
to the number of cell divisions CTLs can undergo in the
absence of CD4⁺ help, a biphasic infection course is
predicted similar to that actually observed in MHC cl to the number of cell divisions CTLs can undergo in the absence of CD4⁺ help, a biphasic infection course is predicted similar to that actually observed in MHC class predicted similar to that actually observed in MHC class
Phil. Trans. R. Soc. Lond. B (2000)

predicted by a simple mathematical model describing the Figure 5. Basic properties of CTL-induced pathology, as
predicted by a simple mathematical model describing the
basic dynamics between a virus population, its target cells
and a lytic CTL response (Nowak & Bangham 1996). W predicted by a simple mathematical model describing the
basic dynamics between a virus population, its target cells
and a lytic CTL response (Nowak & Bangham 1996). We
plot the total number of target cells (uninfected + in and a lytic CTL response (Nowak & Bangham 1996). We plot the total number of target cells (uninfected + infected) and a lytic CTL response (Nowak & Bangham 1996). We
plot the total number of target cells (uninfected + infected)
at equilibrium, in dependence of the efficacy of cell-mediated
immunity and the replication rate of the viru plot the total number of target cells (uninfected + infe
at equilibrium, in dependence of the efficacy of cell-m
immunity and the replication rate of the virus. We
assume that the virus is non-cytocidal. We define at equilibrium, in dependence of the efficacy of cell-
immunity and the replication rate of the virus. We
assume that the virus is non-cytocidal. We define
immunonathology by a reduction of the total numb immunity and the replication rate of the virus. We
assume that the virus is non-cytocidal. We define
immunopathology by a reduction of the total number of assume that the virus is non-cytocidal. We define
immunopathology by a reduction of the total number of
target cells in the presence of CTL, compared to the absence
of CTL, CTL-induced pathology is most likely to occur at immunopathology by a reduction of the total number of
target cells in the presence of CTL, compared to the absence
of CTL. CTL-induced pathology is most likely to occur at a
low or intermediate efficacy of the CTL response target cells in the presence of CTL, compared to the absence
of CTL. CTL-induced pathology is most likely to occur at a
low or intermediate efficacy of the CTL response. In addition,
the replication rate of the virus plays of CTL. CTL-induced pathology is most likely to occur at a
low or intermediate efficacy of the CTL response. In addition,
the replication rate of the virus plays an important role. The
faster the replication kinetics of th faster the replication rate of the carry of the CTL response. In addition, the replication rate of the virus plays an important role. The faster the replication kinetics of the virus, the more severe the degree of patholog the replication rate of the virus plays an important role. The faster the replication kinetics of the virus, the more severe the degree of pathology observed. If the virus replicates at a fast rate, a significant reduction degree of pathology observed. If the virus replicates at a fast
rate, a significant reduction in the total number of target cells will be observed even in the presence of a relatively strong rate, a significant reduction in the total number of target cel
will be observed even in the presence of a relatively strong
CTL response. If the virus replicates slowly, any degree of
immunopathology is observed only in t will be observed even in the presence of a relatively stre
CTL response. If the virus replicates slowly, any degree
immunopathology is observed only in the presence of
inefficient CTL. Thus, for slowly replicating viruses, immunopathology is observed only in the presence of
inefficient CTL. Thus, for slowly replicating viruses, an immunopathology is observed only in the presence of
inefficient CTL. Thus, for slowly replicating viruses, an
increase in the CTL responsiveness is likely to benefit the
host, while for faster replicating strains, the oppo inefficient CTL. Thus, for slowly replicating viruses, an
increase in the CTL responsiveness is likely to benefit the
host, while for faster replicating strains, the opposite applies
(see vertical dashed line and arrows) host, while for faster replicating strains, the opposite applies (see vertical dashed line and arrows). β = replication rate.

(see vertical dashed line and arrows). β = replication rate.
II-deficient mice (Thomsen *et al.* 1996). Under these
conditions it is also predicted that the time-period until II-deficient mice (Thomsen *et al.* 1996). Under these conditions, it is also predicted that the time-period until virus resurgence is inversely correlated to the replication II-deficient mice (Thomsen *et al.* 1996). Under these conditions, it is also predicted that the time-period until virus resurgence is inversely correlated to the replication rate of the virus Thus mathematical simulation conditions, it is also predicted that the time-period until
virus resurgence is inversely correlated to the replication
rate of the virus. Thus mathematical simulation helps in
identifying replication rate as a central vir virus resurgence is inversely correlated to the replication
rate of the virus. Thus mathematical simulation helps in
identifying replication rate as a central viral parameter
that significantly affects the outcome of a vir rate of the virus. Thus mathematical simulation helps in identifying replication rate as a central viral parameter that significantly affects the outcome of a viral infection even under conditions where primary T-cell cont identifying replication rate as a central viral parameter
that significantly affects the outcome of a viral infection
even under conditions where primary T-cell control may
be obtained that significan
even under co
be obtained.
Similar to

Similar to the analysis of T-cell dynamics under be obtained.

Similar to the analysis of T-cell dynamics under

various conditions, the modelling approach may also be

used to predict parameters critically affecting the degree Similar to the analysis of T-cell dynamics under
various conditions, the modelling approach may also be
used to predict parameters critically affecting the degree
of immunonathology induced by the generated effector T used to predict parameters critically affecting the degree
of immunopathology induced by the generated effector T used to predict parameters critically affecting the degree
of immunopathology induced by the generated effector T
cells. This is very important since with non-cytocidal
viruses this is the factor that determines the life o of immunopathology induced by the generated effector T
cells. This is very important since with non-cytocidal
viruses this is the factor that determines the life or death
of the infected bost. An apparant paradox in this c cells. This is very important since with non-cytocidal
viruses this is the factor that determines the life or death
of the infected host. An apparant paradox in this context
is the observation that a partial immune defect viruses this is the factor that determines the life or death
of the infected host. An apparant paradox in this context
is the observation that a partial immune defect may of the infected host. An apparant paradox in this context
is the observation that a partial immune defect may
sometimes be associated with augmented immuno-
pathology (see Marker et al. 1976; Leist et al. 1989). The is the observation that a partial immune defect may
sometimes be associated with augmented immuno-
pathology (see Marker *et al.* 1976; Leist *et al.* 1989). The
reason for this becomes obvious when considering that a sometimes be associated with augmented immuno-
pathology (see Marker *et al.* 1976; Leist *et al.* 1989). The
reason for this becomes obvious when considering that a
weakening of the T -cell response not only reduces the pathology (see Marker *et al.* 1976; Leist *et al.* 1989). The reason for this becomes obvious when considering that a weakening of the T-cell response not only reduces the capacity to induce tissue damage, but also impai reason for this becomes obvious when considering that a weakening of the T-cell response not only reduces the capacity to induce tissue damage, but also impairs anti-
viral effector capacity. As a consequence of the latter, the
infection is less efficaciously contained, allowin capacity to induce tissue damage, but also impairs anti-
viral effector capacity. As a consequence of the latter, the
infection is less efficaciously contained, allowing more
host cells to become infected and thus potentia viral effector capacity. As a consequence of the latter, the infection is less efficaciously contained, allowing more host cells to become infected and thus potential targets for the immune attack infection is less efficaciously contained, allowing more
host cells to become infected and thus potential targets for
the immune attack.
When this is analysed in mathematical terms (figure 5), host cells to become infected and thus potential targets for

it becomes evident that immunopathology is to be

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expected at intermediate levels of T-cell responsiveness
and that the rate of viral replication plays an important expected at intermediate levels of T-cell responsiveness
and that the rate of viral replication plays an important
role in determining the extent as well as the precise expected at intermediate levels of T-cell responsiveness
and that the rate of viral replication plays an important
role in determining the extent as well as the precise
conditions under which immunonathology may be and that the rate of viral replication plays an important
role in determining the extent as well as the precise
conditions under which immunopathology may be
observed This fits reasonably well with our observations role in determining the extent as well as the precise
conditions under which immunopathology may be
observed. This fits reasonably well with our observations
in wild-type mice versus IFN-y-deficient mice versus conditions under which immunopathology may be observed. This fits reasonably well with our observations in wild-type mice versus IFN- γ -deficient mice versus observed. This fits reasonably well with our observations
in wild-type mice versus IFN-γ-deficient mice versus
mice deficient in both IFN-γ and perforin (Nansen et al.
1999: A R Thomsen unpublished observation) Thus if in wild-type mice versus IFN-γ-deficient mice versus
mice deficient in both IFN-γ and perforin (Nansen et al.
1999; A. R. Thomsen, unpublished observation). Thus, if
LCMV Armstrong is used for infection no immunomice deficient in both IFN- γ and perforin (Nansen et al.
1999; A. R. Thomsen, unpublished observation). Thus, if
LCMV Armstrong is used for infection, no immuno-
nathology is associated with infection of wild-type mice 1999; A. R. Thomsen, unpublished observation). Thus, if LCMV Armstrong is used for infection, no immuno-
pathology is associated with infection of wild-type mice.
Eliminating IEN- γ has little effect, whereas lack of bo LCMV Armstrong is used for infection, no immuno-
pathology, precise analysis of normal polyclonal T-cell
pathology is associated with infection of wild-type mice.
Eliminating IFN- γ has little effect, whereas lack of bot pathology is associated with infection of wild-type mice.
Eliminating IFN- γ has little effect, whereas lack of both
IFN- γ and perforin is associated with high (CD8⁺ T-cell-
mediated) mortality (ca. 90%). In contra Eliminating IFN- γ has little effect, whereas lack of both IFN- γ and perforin is associated with high (CD8⁺ T-cell-
mediated) mortality (*ca*. 90%). In contrast, following
infection with LCMV Traub, transient immun mediated) mortality $(a. 90\%)$. In contrast, following infection with LCMV Traub, transient immunopathology mediated) mortality (*ca.* 90%). In contrast, following
infection with LCMV Traub, transient immunopathology
is induced even in wild-type mice and elimination of
IFN- γ results in high mortality (*ca.* 85%). However add infection with LCMV Traub, transient immunopathology
is induced even in wild-type mice and elimination of
IFN- γ results in high mortality (*ca*. 85%). However, addi-
tional elimination of perforin markedly reduces immun IFN- γ results in high mortality (*ca*. 85%). However, additional elimination of perforin markedly reduces immuno-IFN- γ results in high mortality (*ca.* 85%). However, additional elimination of perforin markedly reduces immuno-
pathology (< 15%). Thus with the exception of very low
mortality in Traub-infected double knockout mice, tional elimination of perforin markedly reduces immuno-
pathology $(< 15\%$). Thus with the exception of very low
mortality in Traub-infected double knockout mice, the
experimental observations fit the predictions well. Th pathology $\langle \leq 15\% \rangle$. Thus with the exception of very low
mortality in Traub-infected double knockout mice, the
experimental observations fit the predictions well. The
reason for the latter discrepancy is probably tha mortality in Traub-infected double knockout mice, the experimental observations fit the predictions well. The reason for the latter discrepancy is probably that with a experimental observations fit the predictions well. The
reason for the latter discrepancy is probably that with a
rapidly replicating virus, very reduced virus control leads
to overwhelming infection, which not only increa reason for the latter discrepancy is probably that with a
rapidly replicating virus, very reduced virus control leads
to overwhelming infection, which not only increases the
number of target cells (i.e. risk of immunonatho rapidly replicating virus, very reduced virus control leads
to overwhelming infection, which not only increases the
number of target cells (i.e. risk of immunopathology), but
also, rapidly, exhausts, the host, response, po to overwhelming infection, which not only increases the number of target cells (i.e. risk of immunopathology), but also rapidly exhausts the host response potential (i.e. number of target cells (i.e. risk of immunopathology), but
also rapidly exhausts the host response potential (i.e.
further reduces T-cell effector capacity). We are presently
testing whether this explanation holds true in also rapidly exhausts the host response potential (i.e. further reduces T-cell effector capacity). We are presently testing whether this explanation holds true *in vivo*. To further evaluate the use of mathematical modelli

testing whether this explanation holds true *in vivo*.
To further evaluate the use of mathematical modelling as a tool to predict the outcome of viral infection, we To further evaluate the use of mathematical modelling
as a tool to predict the outcome of viral infection, we
included into the basic model the assumptions that
(i) generation of CTLs is independent of the presence of as a tool to predict the outcome of viral infection, we
included into the basic model the assumptions that
(i) generation of CTLs is independent of the presence of
IFN- γ and (ii) cytokine production contributes to included into the basic model the assumptions that

(i) generation of CTLs is independent of the presence of

IFN- γ , and (ii) cytokine production contributes to

inhibition of viral replication Under these assumptions (i) generation of CTLs is independent of the presence of IFN- γ , and (ii) cytokine production contributes to inhibition of viral replication. Under these assumptions (Bartholdy *et al.* 2000) the model predicts that the IFN- γ , and (ii) cytokine production contributes to inhibition of viral replication. Under these assumptions (Bartholdy *et al.* 2000), the model predicts that the outcome of infection in IFN- γ -deficient mice is crit inhibition of viral replication. Under these assumptions (Bartholdy *et al.* 2000), the model predicts that the outcome of infection in IFN- γ -deficient mice is critically dependent on the replication rate of the virus (Bartholdy *et al.* 2000), the model predicts that the outcome of infection in IFN- γ -deficient mice is critically dependent on the replication rate of the virus and that persistent infection in the absence of substanti outcome of infection in IFN-y-deficient mice is critically
dependent on the replication rate of the virus and that
persistent infection in the absence of substantial immuno-
pathology is only possible for slowly replicatin dependent on the replication rate of the virus and that
persistent infection in the absence of substantial immuno-
pathology is only possible for slowly replicating strains.
Moreover in the latter situation the balance bet persistent infection in the absence of substantial immuno-
pathology is only possible for slowly replicating strains.
Moreover, in the latter situation the balance between
virus load and CTI activity will settle at a new e pathology is only possible for slowly replicating strains.
Moreover, in the latter situation the balance between
virus load and CTL activity will settle at a new equili-Moreover, in the latter situation the balance between
virus load and CTL activity will settle at a new equili-
brium characterized by a compensatory increase in CTL
activity, which is precisely what we observe in IFN- α ristian is band and CTL activity will settle at a new equilibrium characterized by a compensatory increase in CTL activity, which is precisely what we observe in IFN-γ-
deficient mice infected with LCMV Armstrong.
Overall activity, which is precisely what we observe in IFN- γ -
deficient mice infected with LCMV Armstrong.
Overall, the apparent success of mathematical modelling

deficient mice infected with LCMV Armstrong.
Overall, the apparent success of mathematical modelling
in predicting experimental outcomes suggests that simula-
tion may be used as a tool to reveal critical parameters of Overall, the apparent success of mathematical modelling
in predicting experimental outcomes suggests that simula-
tion may be used as a tool to reveal critical parameters of
both host and virus, and therefore may beln in t in predicting experimental outcomes suggests that simulation may be used as a tool to reveal critical parameters of both host and virus, and therefore may help in the designing of better-focused and more informative experi tion may be used as a tool to reveal critical parameter
both host and virus, and therefore may help in the desig
of better-focused and more informative experiments. of better-focused and more informative experiments.
8. CONCLUDING REMARKS

For about a decade the study of immunobiology has **below a concluding KEWARKS**
been dominated by a reductionistic view, analysing
immune responses primarily at the single-cell level. This For about a decade the study of immunobiology has
been dominated by a reductionistic view, analysing
immune responses primarily at the single-cell level. This
has clearly provided very important qualitative informabeen dominated by a reductionistic view, analysing
immune responses primarily at the single-cell level. This
has clearly provided very important qualitative informa-
tion about cell subtypes and molecular mechanisms the single-cell level. This
has clearly provided very important qualitative informa-
tion about cell subtypes and molecular mechanisms.
However, our understanding of important quantitative has clearly provided very important qualitative information about cell subtypes and molecular mechanisms.
However, our understanding of important quantitative
issues is still incomplete, and since many features of a tion about cell subtypes and molecular mechanisms.
However, our understanding of important quantitative
issues is still incomplete, and since many features of a However, our understanding of important quantitative issues is still incomplete, and since many features of a
normal immune response are of a quantitative nature,
insight into the cell dynamics involved is a prerequisite
for the understanding of the normal physiology of the formal immune response are of a quantitative nature,
insight into the cell dynamics involved is a prerequisite
for the understanding of the normal physiology of the
immune system. Until recently, it has been difficult to insight into the cell dynamics involved is a prerequisite
for the understanding of the normal physiology of the
immune system. Until recently it has been difficult to
analyse immune responses in precise quantitative terms for the understanding of the normal physiology of the

there was no methodology to reveal real numbers of
antigen-specific T cells. However, with the advent of there was no methodology to reveal real numbers of
antigen-specific T cells. However, with the advent of
recent technology making possible direct visualization there was no methodology to reveal real numbers of
antigen-specific T cells. However, with the advent of
recent technology making possible direct visualization
of antigen-specific cells either through staining with antigen-specific T cells. However, with the advent of
recent technology making possible direct visualization
of antigen-specific cells either through staining with
MHC-tetramers (Altman et al. 1996; Murali-Krishna et recent technology making possible direct visualization
of antigen-specific cells either through staining with
MHC–tetramers (Altman *et al.* 1996; Murali-Krishna *et*
 gl 1998) or detection of cytokine production at the si of antigen-specific cells either through staining with MHC–tetramers (Altman *et al.* 1996; Murali-Krishna *et al.* 1998) or detection of cytokine production at the single-MHC–tetramers (Altman *et al.* 1996; Murali-Krishna *et al.* 1998) or detection of cytokine production at the single-
cell level (Butz & Bevan 1998; Murali-Krishna *et al.* 1998) things are changing fast. Through the use o *al.* 1998) or detection of cytokine production at the single-
cell level (Butz & Bevan 1998; Murali-Krishna *et al.*
1998), things are changing fast. Through the use of this
methodology precise analysis of normal polyclo cell level (Butz & Bevan 1998; Murali-Krishna *et al.* 1998), things are changing fast. Through the use of this methodology, precise analysis of normal polyclonal T-cell responses in the context of several infections has 1998), things are changing fast. Through the use of this methodology, precise analysis of normal polyclonal T-cell
responses in the context of several infections has recently
been published (e.g. Murali-Krishna *et al.* 1998; Belz *et al.*
1998; Busch *et al.* 1998; Flynn *et al* been published (e.g. Murali-Krishna *et al.* 1998; Belz *et al.* 1998; Busch *et al.* 1998; Flynn *et al.* 1998; Hoshino *et al.* 1999; Stevenson *et al.* 1998). Although these studies in themselves provide crucial informa 1998; Busch *et al.* 1998; Flynn *et al.* 1998; Hoshino *et al.* 1999; Stevenson *et al.* 1998). Although these studies in themselves provide crucial information, no doubt much more information on cell dynamics can be ext 1999; Stevenson *et al.* 1998). Although these studies in themselves provide crucial information, no doubt much more information on cell dynamics can be extracted at this stage through a collaborative effort of experiment themselves provide crucial information, no doubt much
more information on cell dynamics can be extracted at
this stage through a collaborative effort of experimental
immunologists and theoretical biologists. Thus combining more information on cell dynamics can be extracted at this stage through a collaborative effort of experimental immunologists and theoretical biologists. Thus combining this stage through a collaborative effort of experimental
immunologists and theoretical biologists. Thus combining
precise quantitative analysis with mathematical model-
ling should lead to a rapid advancement in our perce immunologists and theoretical biologists. Thus combining
precise quantitative analysis with mathematical model-
ling should lead to a rapid advancement in our percept-
ion of important issues such as regulation of lymphocy ling should lead to a rapid advancement in our perception of important issues such as regulation of lymphocyte homeostasis, memory and immune exhaustion.

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