# Transgenic »knock-out« mice used in the study of the immunology of infectious diseases.

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*Introduction*

Selective breeding combining traits from different inbred mouse strains has for a long time been the only way to deliberately achieve genetically altered mice. Alternatively, screening for spontaneous mutations affecting critical genes has been used. The availability of inbred mouse strains differing in major histocompatibility complex (MHC, H-2 in the mouse) genes has been of great value when studying infection immunology, and results obtained in such mice constitute an important part of the foundation for current thinking in immunology. Thus, through comparison of H-2 congenic mouse strains, T-cell responsiveness against several infectious organisms has been found to be influenced by MHC genes (Doherty et al. 1978, Zinkernagel et al. 1978, Zinkernagel et al. 1985, Kast et al. 1986, Chesebro et al. 1990, Brett et al. 1992), and similar comparisons of H-2 identical, non-H-2 different mouse strains have clearly pointed to an immunoregulatory role also of non-MHC genes (Zinkernagel et al. 1985, Thomsen & Marker 1989, Christensen et al. 1994b). In the case of mutations emerged in inbred mice, both T-cell deficient nude mice and T- and B-cell deficient SCID mice have proved to be important tools to study involvement of either T or B cells or T-cell subpopulations in the defence against infectious organisms. Such mice may be used either directly to demonstrate the need for major lymphocyte populations or as reaction chambers in adoptive transfer experiments where these mice can be selectively reconstituted with relevant subpopulations. Finally, more specific mutations such as the loss mutation of the $L^d$ locus in BALB/c-H-$2^{dm2}$, has been used extensively to demonstrate the importance of a specific MHC class I allele (Ciavarra & Forman 1982, Örn et al. 1982, Allan & Doherty 1985, Thomsen & Marker 1989). In contrast to mice with targeted gene disruption (»knock-out« mice) in which a large sequence of base-pairs is changed, a spontaneous mutation is typically a point mutation which much more easily may revert to a functional sequence. Therefore, some drawbacks must always by expected when using mice with a spontaneous mutation. The SCID mouse is a classical example of this, since these mice, as they grow older, tend to become leaky, producing low, but significant numbers of B and T cells which express a functional antigen receptor, and as such form an oligoclonal receptor repertoire (Bosma et al. 1988). Such leakiness which is partly the result of reversion of the mutation in individual lymphocytes results in changes of the »phenotype«. Two other drawbacks are that the mutated gene may not be precisely known, and that the genetic defect may be pleiotropic which clearly must be taken into consideration when conclusion are to be drawn from experiments with such mice. However, the disadvantages of the SCID mouse have apparently been overcome with generation of the RAG-1 (Mombaerts et al. 1992) and RAG-2 (Shinkai et al. 1992) deficient mouse strains. In both strains the ability to perform V(D)J recombination of both B and T receptor genes has been eliminated by targeted gene disruption, and in contrast to the SCID mouse, observations made to date has not demonstrated leakiness of either strain (Mombaerts et al. 1992, Shinkai et al. 1992), thus demonstrating one of

the advantages of deliberate gene disruption. The »knock-out« technology has been extensively applied in mice, and especially within the field of immunology, since the immune system of the mouse is well characterized and in most cases show close resemblance to the human immune system. Below, examples of how »knock-out« mice have been used to improve our knowledge of how the host handles infectious diseases, will be presented.

*T-Cell Compatments*

T lymphocytes constitute one of the major classes of lymphocytes. These cells derive from precursors found in hematopoietic tissue, which migrate to the thymus and here undergo differentiation and maturation to immunocompetent, naive T cells. These are then seeded to the peripheral lymphoid tissues and join the recirculating pool of lymphocytes.

The T cell antigen receptor (TCR) composition divides T cells into two broad categories. The majority of T cells express antigen receptors composed of $\alpha$ and $\beta$ chains while a minority T cell population expresses TCRs consisting of $\gamma$ and $\delta$ chains. During thymic ontogeny rearrangement of $\gamma$- and $\delta$- chains precede that of $\alpha$- and $\beta$- chains, but it has been unclear whether the development of $\gamma\delta$ T cells influence the development of $\alpha\beta$ T cells and vice versa. However, in TCR$\delta$ mutant mice no surface expression of any $\gamma\delta$ gene products can be detected, yet this defect does not seem to influence the development of $\alpha\beta$ T cells (Itohara et al. 1993). Similarly, development of $\gamma\delta$ cells does not seem to be affected by either TCR$\alpha$ or $\beta$ gene disruption (Mombaerts et al. 1992).

In contrast to B cells, T cells fail to recognize free antigenic determinants. Rather, the TCR recognize a complex consisting of a peptide fragment associated with major histocompatibility complex (MHC) protein. The MHC class II molecule is composed of an $\alpha$ and $\beta$ chain which are both required for antigen presentation to T cells expressing the CD4 coreceptor. In contrast, peptide presentation to CD8 coreceptor expressing T cells by MHC class I occurs through a single $\alpha$ chain, the stabilization of which, however, depends on association with $\beta_2$-microglobulin ($\beta_2$m). Importantly, the conventional MHC class I-restricted $CD8^+$ T cells and the MHC class II-restricted $CD4^+$ T cells are missing in $\beta_2$m-/- (Zijlstra et al. 1990) or A$\beta$-/- (Cosgrove et al. 1992) mutants, respectively, because development of these T-cell subsets depends exclusively on their positive selection through interaction with their respective MHC molecules in the thymus. Furthermore, the importance of CD4 and CD8 molecules in the process of positive selection in the thymus was first demonstrated in mice treated with monoclonal antibodies, and later these findings have been confirmed in transgenic mice lacking surface expression of CD4 (Rahemtulla et al. 1991) or CD8$\alpha$ (Fung-Leung el at. 1993). Thus, MHC class I and II as well as CD4 and CD8 deficient mice are important tools in investigating the involvement of separate T cell subsets during infection. However, one should keep in mind that several minor populations – MHC-independent $\alpha\beta$ T cells expressing the CD8 $\alpha\alpha$ homodimer, MHC-independent $CD4^-CD8^-$ $\alpha\beta$ TCR cells and MHC-independent $CD4^-$ $CD8^-$ $\gamma\delta$ T cells – have been found in $\beta_2$m-/- or A$\beta$-/- mice. The role of these cell types in exerting compensatory functions clearly must be taken into consideration when experimental results are to be interpreted. While this at first sight can be considered a nuisance, it does at the same time make knock-out mice excellent for analyzing functional redundancy within the immune system.

*T Cell Subsets in Viral Infections*

Viral infections are generally held to be eliminated by $CD8^+$ T cells; thus, studies of several viral infections: influenza A virus, Sendai virus, murine cytomegalovirus (MCMV) and lymphocytic choriomeningitis virus (LCMV) have consistently pointed to the importance of $CD8^+$ T cells for virus clearance. However, the importance of $CD4^+$ and $CD8^+$

T cells seem to vary with the infection. Thus, when the $CD4^+$ subset is depleted with monoclonal antibody (Mab), LCMV (Leist et al. 1987, Moskophidis et al. 1987, Ahmed et al. 1988), influenza A virus (Lightman et al. 1987) and Sendai virus (Hou et al. 1992) can still be controlled. In contrast, MCMV infection (Jonjic et al. 1989) can be eliminated from most organs but not the salivary glands in the absence of $CD4^+$ T cells. The differences are even more striking when the effect of $CD8^+$ T-cell depletion is compared. Such depletion result in complete inability to clear LCMV (Moskophidis et al. 1987), whereas mice infected with influenza A virus- (Eichelberger et al. 1991), VSV- (Jonjic et al. 1989), Sendai virus- (Hou et al. 1992) and MCMV-infected (Jonjic et al. 1990) are still able to terminate these infections. Thus, even though $CD8^+$ T cells are held to be the most important T-cell subset in virus clearance, the results mentioned above indicate that both major T-lymphocyte subset may exert effector functions important for the elimination of viral infections. However, the creation of $\beta_2$m-/- and Aβ-/- mice has allowed this important question to be reexamined using an independent method to obtain selective depletion in vivo.

Although influenza virus is not a natural mouse pathogen, mice infected intranasally with this virus develop a temporary viral pneumonia, the severity of which varies with the isolate. $\beta_2$m-/- mice have been found to clear some strains of influenza A virus within the same time frame as do $\beta_2$m+/+ control mice (Eichelberger et al. 1991). However, infection with more virulent virus strains tends to result in substantial mortality in $\beta_2$m-/- mice (Bender et al. 1992), probably because the compensatory mechanisms acting in these mice become overburdened. Since depletion of $CD4^+$ cells in normal mice also results in little delay in virus clearance (Eichelberger et al. 1991), and because T-cell deficient nude mice die from infection with this virus (Scherle et al. 1992), these results suggest that both $CD8^+$ and $CD4^+$ T cells can independently mediate the clearance of influenza virus in the mouse, albeit with slightly different efficiency.

Another commonly studied viral infection is vaccinia virus which, however, also is unnatural to the murine host. Infection with this virus generally leads to a temporary infection in normal mice and lethal infection in T-cell deficient nude mice (Spriggs et al. 1992). When $\beta_2$m-/- mice were infected intradermally with this virus, visible lesions as well as the course of their healing was found to follow a similar pattern in $\beta_2$m-/- mice and wildtype controls (Spriggs et al. 1992). Notably no generalized spread of viral lesions was noted in any group, and weight gain was unaffected by the infection. However, since viral titers were not followed, the conclusions that can be drawn from this study are limited, except that compensatory mechanisms saved these animals from a lethal outcome. However, a surprising observation was made in $\beta_2$m-/- mice concerning the secondary IgG antibody response to vaccinia virus infection. Even through $CD8^+$ T cells are not known to play significant role in promoting antibody responses, $\beta_2$m-/- mice infected with vaccinia virus produced a substantially weaker IgG response than did $\beta_2$m+/- mice (Spriggs et al. 1992). The total levels of IgG isotypes in $\beta_2$m-/- mice were also reduced significantly compared to controls, even before immunization. One possibility is that $\beta_2$m-/- mice have an altered cytokine profile due to the absence of $CD8^+$ T cells. Given that $CD8^+$ cells are known to produce substantial amounts of interferon-γ, which has been implicated in regulating Ig class switching, lack of this cytokine may explain the reduced IgG level. It should be noted, however, that all IgG isotypes tested were affected whereas only selected isotypes are known to be positively regulated by interferon-γ.

The murine parainfluenza type 1 virus, Sendai, is a natural respiratory pathogen in the mouse. Various studies have suggested that class I restricted $CD8^+$ T cells play a critical role in clearing Sendai virus (Kast et al. 1986)

and, indeed, when $\beta_2$m-/- mice were infected with this virus, they presented a significantly delayed clearance and an increased mortality compared to wildtype littermates (Hou et al. 1992). In this model, it was further shown that CD4+ T cells served as a critical back-up system, because $\beta_2$m-/- mice depleted of CD4+ cells succumbed to the infection whereas the majority of intact $\beta_2$m-/- mice survived.

Theilers virus is a murine picornavirus that may infect the central nervous system and induce a chronic demyelinating disease in susceptible mice (Rodriguez et al. 1987) resembling multiple sclerosis in man. It has been proposed that CD8+ T cells play a pivotal role in the pathogenesis as well as the clearance of this viral infection. When $\beta_2$m-/- mice were infected with Theilers virus, it became clear that these mice fail to clear the virus (Fiette et al. 1993), but in contrast to expectations do develop demyelinating disease (Fiette et al. 1993, Pullen et al. 1993, Rodriguez et al. 1993), suggesting that CD8+ T cells may not be the direct cause of demyelination, as was previously thought.

Infection with lymphocytic choriomeningitis virus (LCMV), a natural arenavirus of the mouse, may in adult, immunocompetent mice take one of two courses depending on the route of inoculation. Intracerebral (i.c.) injection mostly initiates a fatal choriomeningitis 6-8 days post infection (p.i.) which is absent in T-cell deficient mice (Christoffersen et al. 1976, Leist et al. 1987, Andersen et al. 1991), and which has been shown to be mediated by virus-specific T cells (Cole et al. 1972, Doherty & Zinkernagel 1975). By contrast, inoculation by a peripheral route generally causes little or no disease, but elicits an antiviral immune response which eventually clears the virus from all organs (Moskophidis et al. 1987).

Studies involving adoptive transfer of antigen-primed effector cells as well as in vivo depletion experiments with Mabs against CD4 and CD8, have established that regardless of route of infection, the induction of a MHC class I-restricted CD8+ response is critical to the outcome of infection (Doherty et al. 1976, Zinkernagel & Welsh 1976, Dixon et al. 1987, Leist et al. 1987, Moskophidis et al. 1987) whereas CD4+ T cells do not seem to be crucially involved. Furthermore, consistent with this, immunogenetic studies have revealed a strong Ir-gene effect exerted by MHC class I genes (reviewed in Thomsen & Pfau 1993).

Several groups, including our own, have undertaken to investigate the involvement of CD8+ T cells in LCMV infection using »knock-out« mice. When $\beta_2$m -/- mice are infected i.c. with LCMV, this leads to a less severe disease compared to $\beta_2$m +/+ mice. The disease in $\beta_2$m -/- mice is characterized by low grade inflammation in CSF (Doherty et al. 1993b, Christensen et al. 1994a), general wasting (Doherty et al. 1993b, Lehmann-Grube et al. 1993), and some mortality (Muller et al, 1992, Christensen et al. 1994a). Although the severity of the infection varies in different studies, probably due to the virus isolate used, all groups agree that the disease in $\beta_2$m -/- mice is less severe than that seen in wildtype littermates and without the classical symptoms associated with LCM disease. Furthermore, all of the studies indicate that the milder disease observed in $\beta_2$m -/- mice, contrary to expectations, is mediated by LCMV-specific CD4+ T cells.

The mechanisms by which CD4+ T cells causes disease in i.c. infected animals is uncertain. Class II restricted and LCMV-specific CD4+ CTLs were detected in $\beta_2$m -/- mice in two studies (Hou et al. 1992, Muller et al, 1992), but not in a third (Lehmann-Grube et al.1993). Although there are relatively few class II-positive cells that could serve as target cells in normal brain tissue, upregulation of class II expression has been demonstrated, and virus-specific CD4+ CTLs must clearly be taken into consideration. Another possibility is that the disease is mediated indirectly by cytokine-producing CD4+ T cells. If this is the case, the disease is likely to reflect a direct effector function of CD4+ T cells ra-

ther than an antibody mediated reaction, since previous studies in antibody-deficient mice have failed to implicate these, at least in conventional LCM disease.

When $\beta_2$m -/- mice are infected intravenously with LCMV, high organ virus titers are maintained for several month in contrast to complete clearance within 2-3 weeks in wildtype animals (Lehmann-Grube et al.1993). In parallel to the finding of a CD4+ T-cell mediated response in i.c. infected animals, studies in our lab have shown that virus titers in certain organs are reduced due to a CD4+ T-cell dependent reaction (Christensen et al. 1994a). Thus, when $\beta_2$m -/- mice were compared to T-cell deficient nude mice infected in parallel, significantly reduced spleen virus titers were observed three weeks post infection, and depletion of CD4+ T cells partly reversed this situation.

In conclusion, these findings strongly indicate that class I expression and a CD8+ T-cell mediated immune response is essential for clearing the LCMV infection. Furthermore, the study in $\beta_2$m -/- mice underscores the value of »knock-out« mice, since a CD4+ CTL response is only to be found in absence of CD8+ T cells, and, finally, it demonstrates how compensatory mechanisms may arise in absence of a specific cell subset and may be analyzed.

Using Aβ -/- mice, we have also undertaken to investigate to the extent to which CD4+ T cells are needed for the activation and maintenance of the CD8+ T-cell mediated immunity. From these studies, it is evident that CD4+ T cells are not required in the induction of virus specific CD8+ T cells in that CD8+ T cells are equally activated and responsive in terms of adhesionmolecule expression, DTH-reactivity and CTL activity (Christensen et al. 1994a). However, very interesting and surprising results were obtained when restimulation of LCMV-specific CTL activity in vivo was analyzed. In this case, Aβ -/- mice showed no evidence of CD8+ CTL memory 60 days post infection whereas wild-type mice gave a marked response (Christensen et al. 1994a). Furthermore, it was found that viremia reappeared in Aβ -/- mice around this time point, indicating that CD4+ T cells are required for long-term CD8+ cell mediated immunsurveillance. Further studies are needed, however, to determine in which phase of the immune response CD4+ T cells are important: whether CD8+ memory, but not effector cell generation depends on the presence of CD4+ T cells, or CD4+ cells are required for long-term maintenance of CD8+ activity.

*T Cell Subsets in Bacterial Infections*

Although protective immunity against intracellular bacteria and protozoa was originally considered to exclusively depend on MHC class II-restricted CD4+ T cells, strong evidence now exists for involvement of MHC class I-restricted CD8+ T lymphocytes (Kaufmann 1993). This is most clearly demonstrated for Listeria monocytogenes in which case CD8+ T cell are of major importance in clearing a primary infection as well as in resistance to reinfection (Mielke et al. 1988). L. monocytogenes is known to escape into the cytoplasm of infected cells, thus making the involvement of class I-restricted T cells predictable. However, evidence for CD8+ T cell participation has also been found in murine infection with Mycobacterium tuberculosis (Orme & Collins 1984) which is believed to reside predominantly in the endosomal compartment, thus suggesting that endosomal protein may leak into the class I presentation pathway. In contrast, avirulent M. bovis is generally considered the model pathogen for exclusive CD4+ T-cell contribution to immunity.

Infections with M. tuberculosis and L. monocytogenes are markedly exacerbated in $\beta_2$m -/- mice (Flynn et al. 1992, Roberts et al. 1993), emphasizing the central role of CD8+ T cells in the control of these infections. In M. tuberculosis infected $\beta_2$m -/- mice this leads to a lethal infection in contrast to the situation in wild type littermates (Flynn et al. 1992). M. bovis infection, however, failed to cause a lethal infection in these mice.

The findings in M. tuberculosis and L. monocytogenes infections strongly support a role for $CD8^+$ T cells in the antibacterial immune defence. Since M. tuberculosis, M. bovis and L. monocytogenes infections are also exacerbated in Aβ -/- mice (Kaufmann 1994) the overall picture is consistent with the assumption that optimum protection against murine listeriosis and tuberculosis depends on both $CD4^+$ and $CD8^+$ T cells.

With regard to the function of αβ- and γδ- T cells, the important role of αβ T cells has been clearly established whereas the functional importance of γδ T cells has been more uncertain. In an investigation of the relative roles of these T-cell populations using TCR knock-out mice, it was found that in primary listeriosis, either αβ or γδ T cells are sufficient for early protection (Mombaerts et al. 1993). However, resistance to secondary infection with Listeria is mediated mainly by αβ T cells but also involves γδ T cells which may partly compensate for lack of αβ T-cells. Thus, αβ T-cell deficient mice can be rendered partially resistant by vaccination, and γδ T cells have been shown to be responsible for this protective effect (Mombaerts et al. 1993). Furthermore, in contrast to the development of granulomatous lesions mediated by αβ T cells, γδ T cells are not sufficient for this expression of antibacterial immunity. However, γδ T cells appear to modulate the activity of αβ T cells so that in their absence numerous abscess-like lesions are found rather than normal granulomatous lesions. Thus, TCR mutant mice provide important tools for the identification of functions unique to αβ and γδ T cells and, for the study of their interplay.

*Cytokines and Adhesionmolecules*

The cytokines required to support appropriate defence mechanisms have been identified in several bacterial and parasitic infections to depend critically on $CD4^+$ T cells. $CD4^+$ T cells may be divided into two major types, T helper type 1 ($T_h1$) and T helper type 2 ($T_h2$) cells, defined by their cytokine production profile (Mosmann et al. 1989). $T_h1$ clones mainly produce IL-2, IFN-γ and TNF-β, but not IL-4, IL-5 or IL-10. Conversely $T_h2$ cells produce IL-4, IL-5 and IL-10, but not IL-2, IFN-γ and TNF-β. The type of $T_h$ cell which dominate a given immune response seems to be regulated partly by cytokines of the innate defence system (e.g. IFN's, IL-12) and to be further fixed by cytokines produced in the context of the specific immune response. Thus, a delicate interplay exist between the $T_h1$ and $T_h2$ subsets in that IFN-γ inhibits $T_h2$ clones and IL-10 inhibits $T_h1$ clones, and autocrine production of IL-2 and IL-4 promotes expansion of $T_h1$ and $T_h2$ clones, respectively. In addition to $CD4^+$ cells also $CD8^+$ cell may produce cytokines in response to infectious agents. The profile of their cytokine production seems to match that of $T_h1$ cells (Fong & Mosmann 1990) which may be of importance for the ability of these cells to initiate inflammation and eliminate virus from sites of infection. It is pertinent to note that the spectrum of cytokines produced during an infection may dramatically alter the course of this infection (Louis & Müller 1989). Therefore many studies have been directed to clarify the intricate interaction of known cytokines in relation to many different disease models, and this type of studies has made a big leap forward with the availability of cytokine »knock out« mice.

Assessment of the requirement for IFN-γ has been made possible by generation of both IFN-γ receptor (IFN-γR) (Huang et al. 1993) or IFN-γ deficient mice (Dalton et al. 1993). Of viral infections both vaccinia and influenza virus infections have been used to evaluate the role of IFN-γ. From these studies it is evident that IFN-γ contributes to the regulation of vaccinia infection, since mice lacking IFN-γR demonstrate increased sensitivity and increased virus titers compared to control mice (Huang et al. 1993). In contrast, IFN-γ negative mice are no more sensitive than wildtype littermates to pulmonary challenge with influenza virus (Graham et al.

1993) suggesting no role for IFN-γ during this infection. Taken together, these studies indicate that IFN-γ may be important in some, but not all, viral infections.

Studies with IFN-γ and IFN-γR mutants have confirmed the central role of this cytokine in immunity to intracellular bacteria. From experimental infection with M. bovis in IFN-γ deficient mice (Dalton et al. 1993) and L. monocytogenes in IFN-γR deficient mice (Huang et al. 1993) it is evident that these mice suffer more severe infection than do wildtype controls. In IFN-γ deficient mice this resulted in killing of the mice by a sublethal dose of M. bovis whereas IFN-γR deficient mice infected with L. monocytogenes showed increased susceptibility and increased bacterial load.

The role of TNF has so far only been studied in TNF-R1 -/- mice. Using such mice, it was found that TNF was important in the non-specific effector phase against L. monocytogenes (Pfeffer et al. 1993, Rothe et al. 1993), but also mediated part of the toxicity of LPS. Thus, the role of TNF as a two-edged sword in relation to bacterial infection was clearly underscored in these studies. In contrast, the immune response to LCMV and vaccinia virus appeared uncompromised by this defect (Rothe et al. 1993).

As mentioned previously, interleukins are known to be of importance for the regulation of the immune response and, indeed, experiments with IL-2-, IL-4-, IL-6- and IL-10–«knock-out« mice has already yielded several interesting and surprising results, even though very few infectious models have been tested using these mice. In IL-2 deficient mice, thymic development and peripheral T-cell populations are apparently normal (Schorle et al. 1991). However, most interestingly, CTL responses against LCMV and vaccinia virus are within the normal range (Kündig et al. 1993) which appears to contradict the dogma that IL-2 is the most important interleukin for clonal expansion of antigen-primed T cells. In IL-4 deficient mice, the possibly involvement of a $T_h2$ response against infection with the nematode Nippostrongylus brasiliensis has been tested, and it was found that this cytokine is important for expansion of the $T_h2$ subset and for the production of $T_h2$ derived cytokines in that both IL-5 and IL-10 was significantly reduced during this infection (Kopf et al. 1993).

Interleukin-6 is a multifunctional cytokine that regulates various aspects of the immune response, acute-phase reaction and hematopoiesis. Although a multifunctional cytokine, IL-6 deficient mice develop normally. When analyzed during infection, these mice were incapable of controlling efficiently vaccinia virus and infection with L. monocytogenes (Kopf et al. 1994). The T-cell-dependent antibody response against vesicular stomatitis virus was impaired and, finally, the inflammatory acute-phase response after tissue damage or infection was severely compromised. This lead the authors to conclude that IL-6 is an important in vivo SOS signal which coordinates activities of liver cells, macrophages and lymphocytes.

The last interleukin deficient mice to be mentioned is the IL-10 »knock-out« mouse. This cytokine is thought to play an important role in growth and differentiation of hematopoietic cells, and is a potent suppressor of macrophage and T-cell functions, particular $T_h1$ responses. Again, these mice develop normally and shows normal lymphocyte development and antibody production. However, their growth is severely retarded and they have chronic enterocolitis (Kühn et al. 1993). While this mouse strain has not been tested in infectious disease models, the presence of enterocolitis in these mice, which is only observed in mice with a normal intestinal flora, suggests an important down-regulatory function of IL-10 in the immune response against exogenous antigens, e.g. by controlling the $T_h$-subset balance.

Finally, at the time of writing, four types of adhesionmolecule »knock-out« mice have been engineered lacking CD2 (Evans et al.

1993), ICAM-1 (Sligh et al. 1993, Xu et al. 1994), CD28 (Shahinian et al. 1993) or P-selectin (Mayadas et al. 1993), respectively. Even though these mice has not been studied much in infectious disease models, their existence is worth noticing, because very interesting analysis can be conducted in these mice. Not only will they be of importance for analyzing the cellular interactions taking place between cells of the immune system during the afferent and efferent phase of the immune response, but also such important activities as homing of cells to inflammatory sites can be studied in these animals. This would clearly complement experiments involving antibody blocking which are always tainted by the fact that the antibody might delete or inactivate the relevant subset rather than simply blocking the relevant molecular interaction.

*Effector Functions*

There is ample evidence that $CD8^+$ cytotoxic T cells and natural killer (NK) cells are involved in the elimination of some viruses, in graft rejection, in antitumor immune response and in some autoimmune diseases. Although cell-contact dependent cytolytic activity is the functional hallmark of $CD8^+$ and NK cells in vitro, it has been hotly debated whether lytic activity or secretion of cytokines, or a combination of both, mediate the activity of these cells in vivo (e.g. Martz & Howell 1989). To account for lymphocyte-mediated cytotoxicity, several mechanisms have been suggested (Doherty 1993a), among them the granule exocytosis model, programmed cell death or other target cell intrinsic pathways. Because of its ability to form pores upon polymerization, perforin is a plausible granular molecule to be responsible for cell lysis whereas the serin esterase granzyme B is believed to be involved in induction of DNA fragmentation and apoptosis of target cells.

Both perforin and granzyme B are found in the granules of most $CD8^+$ cytotoxic T cells and NK cells; nevertheless, mice deficient for these products present normal numbers of $CD8^+$ and NK-marker-positive cells (Kägl et al. 1994). In perforin deficient mice, virus-specific and MHC antigen-specific CTL activity against fibroblast target cells is absent, as is NK activity measured in vitro (Kägl et al. 1994). Furthermore, these mice are not able to clear infections with LCMV, and they have reduced capacity to control the growth of certain tumor cells. Together, these result demonstrate that perforin is a main killing mechanism of cytotoxic T cells and NK cells in vitro and a crucial effector mechanism in vivo. In contrast to these results, it has been found that CTL effectors from granzyme B deficient mice are capable of inducing slow release of cytoplasmic content from target cells in vitro whereas the DNA fragmentation typical of CTL induced apotosis is markedly reduced (Heusel et al. 1994). As yet these mice have not been studied in relation to resistance to infectious disease. However, together perforin and granzyme B deficient mice are important study object to analyze the role of cytotoxicity as effector mechanism not only in the antiviral immune response, but also in the context of other intracellular organisms, particularly those that are meet with a $CD8^+$ T-cell response.

*Concluding Remarks*

In the previous sections we have presented several examples of how »knock-out« mice has been used in the field of infectious immunology. Many previous studies have been confirmed using these mice, and several new interesting results have emerged. Undoubtedly, more understanding will come in the future both with the »knock-out« mice already generated, but also with the second generation of »knock-out« mice in which genes are deleted in a tissue-specific and/or development-dependent manner. However, even though the advantages of this technique are multiple, some limitations must also be taken into consideration when interpretating results from experiments with these mice. When the use of »knock-out« mice is com-

pared to more classical antibody based inhibition methods, several advantages become obvious: 1) In general the need for stressful manipulation of the animals, such as thymectomy, splenectomy or multiple injections, is avoided. 2) In most cases more complete depletion of cells or proteins is achieved in »knock-out« mice, and what may remain becomes easier to overcome. 3) When antibody depletion of cells has been used, this has in some instances lead to depletion-resistant cells which, although they possess the phenotype depleted for, can not be eliminated. In other cases cells have been physically eliminated instead of simply blocked in a specific function, and, finally, some cells may be activated instead of blocked by antibody treatment. Clearly these difficulties are overcome in »knock-out« mice. 4) Beside the possibility of analyzing the involvement of a single protein or celltype in the immune response, »knock-out« mice also offer the opportunity to analyze compensatory mechanisms which are not normally seen in intact animals. This may either be responses too weak to be detected in intact animals, or responses mediated by cell populations which may only emerge in the absence of another cellsubset (e.g. $CD4^+$ CTL cells) (Hou et al. 1993). Having mentioned the advantages of using »knock-out« mice, one should, however, always bear in mind that the immune system in genetically altered mice has developed in the absence of signals that might have been given by normal counterparts of the mutated genes. Thus, results from genetically altered mice may not simply reflect the consequence of absent signals (e.g. cytokines) in the acute phase of the immune response, but may equally well reflect the consequence of developmental differences between »knock-out« and wildtype mice; i.e. compensatory mechanisms may have arisen that would never be of relevance in a more physiologic setting. Finally, when experiments in a »knock-out« strain have demonstrated the involvement of a protein or a cellsubset, the answer to the question: »at which point in the course of the host response is the protein or cellsubset needed?« is lost. Thus, some experiments with »knock-out« mice will be limited to determine whether a given protein or cellsubset is required, but not in which phase of the host response, it is required. In spite of these reservations, it remains clear that the availability of mice with targeted disruption of specific genes has already provided immunologists with much new insights into the function of the immune system, and without doubt »knock-out« mice are to be important tools in immunology in the years to come.

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