CELLULAR IMMUNE RECOGNITION AND THE BIOLOGICAL ROLE OF MAJOR TRANSPLANTATION ANTIGENS

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by

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THE ORIGINAL EXPERIMENT ON T-CELL SPECIFICITY

While Immunology began as an attempt to understand immunity against infectious disease, in the 1960s and early 1970s it was largely preoccupied with antibody and T-cell responses against easily available foreign protein antigens or chemically defined small molecules (haptens)[1]. The mechanisms of foreign-organ graft rejection were also intensively studied, although the biological function of the highly polymorphic major transplantation molecules coded by the major histocompatibility complex (MHC, HLA in humans, H-2 in mice)[2,3] was largely unclear. Only a few people studied immunity against infectious agents. The Department of Microbiology at the Australian National University in The John Curtin School of Medical Research in Canberra, headed by G. Ada, was one place where virologists and immunologists worked together on antibacterial and antiviral T-cell-mediated immunity, particularly on the capacity of immunized cytotoxic $CD8^+$ T-cells to destroy either virus infected or allogeneic target cells *in vitro*. Peter C. Doherty from Brisbane, who had returned to Australia from Edinburgh at the end of 1971, was working in this Department. He was interested in inflammatory cell responses in the brain against virus infections. When I arrived in Canberra in early 1973 I started working on *Listeria* with R. V. Blanden, and joined forces with Peter to work on cell-mediated immunity to the lymphocytic choriomeningitis virus (LCMV)[4–6]. I had come from an institution that was involved in establishing the ^{51}Cr release assays by T. Brunner and J. C. Cerottini in Lausanne, Switzerland[7,8], and I therefore attempted to establish the cytotoxicity assay against LCMV in the same way as R. V. Blanden and I. Gardner were using it to monitor cellular immune responses against ectromelia virus (mouse pox) in mice[9]. Because many papers on cytotoxic T-cell responses against LCMV had already been published by various groups[10,11] and since studies had already been carried out on mouse pox in Canberra by R. V. Blanden's group, members of the Department were sceptical that we should and could run with the strong competition. Although we had some initial problems because of our limited experience with LCMV we successfully established the test with some help from I. D. Gardner. We then used this test to find out whether in-

flammatory cells in the cerebrospinal fluid of mice infected intracerebrally with LCMV were cytolytic *in vitro* and whether there was a correlation between cytotoxic T-cell activity and severity of choriomeningitis. P. C. Doherty was very good at collecting a few microliters of cerebrospinal fluid from the cisterna magna of a mouse. I miniaturized the ^{51}Cr release assay to be able to measure the activity of such small numbers of cells in microwell plates. These experiments revealed that cytotoxic T-cells specifically destroying LCMV infected target cells could be found in the cerebral spinal fluid of normal mice, but not in that of nude mice lacking a thymus and T-cells; this implied that T-cells probably also destroyed infected meningeal and ependymal cells *in vivo* and that this was the essential pathogenic mechanism causing lethal choriomeningitis. These findings were published in the *Journal of Experimental Medicine* in March 1973[12]. In this journal a paper by Oldstone, McDevitt and collaborators had just appeared, showing that mice with different major histocompatibility gene complexes differed with respect to their susceptibility to LCM disease after intracerebral infection[13]. We therefore checked whether a correlation existed between the virus specific cytotoxic T-cell activity in mice and their susceptibility to disease. Six to eight mice of each inbred and cross-bred strain available at the school were infected intracerebrally with LCMV. Two of each were sacrificed on day 7 after infection, when the first mice became sick, to test antiviral cytotoxic T-cell activities in spleens (Table 1). The remaining mice were monitored for the development of lethal disease during the next ten days. While all the mice died of choriomeningitis by day 10, surprisingly only some strains of mice generated virus-specific cytotoxic T-cell activity that was measurable in our *in vitro* assay (Table 1). This result either signalled that cytotoxic T-cells had nothing to do with lethal choriomeningitis, or, alternatively, that our test was in some way inadequate. The latter interpretation proved to be correct: We had used mouse L-929 cells infected with LCMV as target cells to assess cytotoxic T-cell activities. This was a fibroblast cell line used by virologists at the John Curtin School of Medical Research to quantitate viruses by a plaque assay[14]. Since this was the only mouse-cell line usually used in the Department, along with a Vero-cell line derived from monkeys or the BHK-cell line derived from hamsters, we chose it to provide target cells in the virus-specific cytotoxic T-cell assays. By chance, and fortunately, the mice that were most commonly used in the Department were of the CBA strain, while the L-cells had been derived from a closely related mouse strain C3H some 50 years earlier. Also by chance, both mice possessed the same MHC-molecules ($H\text{-}2^k$). Our critical test now revealed that LCMV-immune spleen cells from all mice that possessed the $H\text{-}2^k$ haplotype (as do e.g. CBA mice), including cross-breeds with $H\text{-}2^k$ mice, lysed L929 ($H\text{-}2^k$) cells infected with the virus, but did not lyse uninfected targets, or those infected with a third-party virus; all spleen cells derived from immunized mice that were not of the $H\text{-}2^k$ type failed to do so[15].

Two additional experiments carried out within the next few weeks promptly confirmed these findings. It was important to show that LCMV-immune lymphocytes from non-$H\text{-}2^k$ strains of mice were able to lyse LCMV-infected

Table 1. Experiments demonstrating MHC-restriction specificity and positive selection in the thymus for antiviral cytotoxic T-cells.

	Stem cells (MHC, H-2)	Thymus (MHC, H-2)	Other host cells (MHC, H-2)	Virus-specific $CD8^+$ T-cells specific for (MHC, H-2)		Lethal $CD8^+$-T-cell-mediated chorio-meningitis after i.c. infection
I	*Original experiments with normal mice*			k	d	
1.	k	k	k	+	–	100 %
2.	b	b	b	–	–	100 %
3.	d	d	d	–	+	100 %
4.	k/d	k/d	k/d	+	+	100 %
II	*Thymus transplantation to thymus-deficient recipient mice:*					
1.	k/d	–	k/d	–	–	0 %
2.	k/d	k	k/d	+	–1	100 %
3.	k/d	d	k/d	–	+	100 %
4.	d	k	d	–	+	100 %

Normal mice (I) vs. mice lacking a thymus that therefore have no T-cells and were given a thymus graft under the kidney capsule (II), were infected with LCMV intracerebrally. All mice died, except those that had no T-cells (II.1.), because of the lethal $CD8^+$ T-cell-mediated LCMV-specific immunopathology. The fact that mice without functional T-cells survived shows that the noncytopathic LCMV does not cause disease directly.

The LCMV-specific cytotoxic T-cells lysed infected target cells that share MHC-molecules (e.g. I.1.; Immune T-cells from infected CBA mice) ($H\text{-}2^k$) lysed infected L929 fibroblast cells ($H\text{-}2^k$)[15].

The MHC of the thymus determines which MHC is recognised by mature T-cells. The experimental group II.3. shows that ($H\text{-}2^k$ x $H\text{-}2^d$) mice with a thymus $H\text{-}2^d$ generated virus-specific T-cells that lysed virus infected $H\text{-}2^d$ target cells but not infected $H\text{-}2^k$ cells[75]. There are, however, experimental exceptions to this rule (II.4.). Thymus-deficient nude mice ($H\text{-}2^d$) reconstituted with an $H\text{-}2^k$ thymus generated $H\text{-}2^d$-restricted T-cells [76].

target cells of the corresponding MHC-type. This did not prove to be easy, because the other available mouse-cell lines in the Department, such as the $H\text{-}2^d$ mastocytoma P815 or the $H\text{-}2^b$ thymoma EL4, could not be infected with LCMV. Because of my work with R. Blanden on *Listeria*–which infects macrophages and is essentially controlled by cell-mediated activation of macrophages as shown by Mackaness[16]–we tried to use macrophages directly isolated from peritoneal washings of mice as target cells for these tests. Macrophages adhered well to plastic, and could be readily infected and labelled with ^{51}Cr. Proper criss-cross experiments showed that LCMV-immune T-cells from $H\text{-}2^b$ mice lyse LCMV-infected macrophages of $H\text{-}2^b$ origin but not those of other H-2 types, and vice versa. The report on these findings was sent off via J. Humphrey as a letter to *Nature* in early December[15]; it was accepted in January 1974, and was published in April 1974. The first public presentation of our data outside Australia was at a Keystone meeting, in Squaw Valley, attended by A. Cunningham in February and a Brook Lodge meeting attended by G. Ada in March 1974. A letter sent back to Canberra by A. Cunningham summarized data from G. Shearer showing that TNP-specific cytotoxic T-cells lysed syngeneic TNP-lated targets more efficiently than allogeneic TNP-lated targets; these data were submitted to the *European Journal of*

Immunology[17] at about the time our report appeared in *Nature*. Obviously, the two findings had emerged independently.

INTERPRETATIONS OF THE DATA

The biological function of MHC and of transplantation antigens was largely unknown in the early 1970s. Their function was obviously not simply to frustrate transplantation surgeons. Transplantation antigens have been defined by P. A. Gorer[18], and by G. Snell[19], based on the work of C. Little, L. Strong and others, who had developed many inbred strains of mice in order to be able to define the rules of transplantation and rejection of tissue and cell grafts (reviewed by J. Klein[2]). Haematologists, particularly J. Dausset[3] and J. van Rood[20], defined lymphocyte surface antigens in humans as being similar to red blood cell antigens, and called them human lymphocyte antigens (HLA). Once many patients had been typed for their transplantation antigens it became apparent that several disease susceptibilities were somehow linked to the transplantation antigen types. It was revealed in studies by B. Benacerraf[21], and later in great and critical detail by H. McDevitt and coworkers[22,23] and by F. Lilly[24], that inbred strains of guinea pigs and mice differed in their responses to some of the model antigens or tumor studied. Because of the availability of well-defined inbred strains of mice, this was readily mapped to the MHC and even to subregions of the MHC in mice by H. McDevitt et al.[25]. In the early 1970s, transplantation antigens were widely discussed because of these findings. MHC polymorphism was thought either to prevent mutual parasitism or transmission of tumor cells[26], or to prevent viruses or other pathogens from mimicking transplantation antigens and, therefore, from eliminating the species[27-29]; alternatively, it was proposed that transplantation antigens functioned as enzymes or as generators of antibody diversity[30]. A most fascinating proposal had been formulated by H.S. Lawrence in 1959[31]. He proposed that infectious agents complexed with transplantation antigens and formed a (*self* + *x*) complex–a fantastically prophetic view of what was to be found later!

There is no doubt that the experiments, which were to reveal the essential role of MHC and T-cell recognition in all, depended upon the foundation built by tumor and transplantation immunologists. Without inbred and MHC (H-2)–congenic or H-2 mutant mouse strains, as developed by G. Snell[19] and C. Bailey[32] and coworkers, respectively, this problem would not at the time have been accessible to analysis. There is also no doubt that MHC-restricted, T-cell recognition would have been discovered by others, using a different approach, a few years later. This would certainly have happened once cloned effector T-cells were developed by M. Schreier, H. Hengartner, H. von Boehmer and G. Fathman[33,34], when T-cell hybridomas were developed by J. Kappler and R. Marrack, or when T-cell receptors were first successfully analyzed by J. Kappler, P. Marrack and J. Allison[35-37] and then were molecularly defined by M. Davis and T. Mak[38,39].

From our very first experiments showing the double specificity of cytotoxic

T-cells for MHC and virus, we immediately knew that we had discovered something important. Our results were not the only ones that hinted at the biological role of major transplantation antigens, and they fitted several observations made during 1972/73. In addition to the suggestions from cytotoxic T-cell studies with leukemia, ectromelia and LCM viruses[9,40,41,41a], there were the experiments by B. Kindred and D. Shreffler[42], who had reported that H-2-incompatible T-helper cells transfused to T-cell-deficient nude mice were not able to help nude B cells make antibodies. Also, P. J. McCullagh and D. H. Katz, H. Hamaoka and B. Benacerraf had shown that histoincompatible B cells and T-cells were not interacting successfully to produce a good IgM to IgG switch[43,44]. In parallel experiments with inbred strains of guinea pigs, A. S. Rosenthal and E. M. Shevach analyzed antigen-specific proliferative T-cell responses, and found them only when primed T-cells and antigen-presenting cells were from guinea pigs with the same MHC type[45]. Most of these experiments were, however, complicated and difficult to interpret, because the mixing of T-cells and B-cells or antigen-presenting cells of different MHC type initiated allogeneic mixed lymphocyte reactions, resulting in non-specific signals. These findings were, therefore, only accepted with hesitation by immunologists; this changed when our data appeared. The simplicity of the *in vitro* assays, the parallel observation by G. Shearer using TNP[17], its easy reproducibility by R. V. Blanden and I. D. Gardner using ectromelia virus[46], by U. Koszinowski using vaccinia virus[47], by E. Simpson, S. Gordon and H. von Boehmer using the male H-Y-antigen[48,49] and by M. J. Bevan using various minor histocompatibility antigens[50], all helped to convince immunologists of the general character of MHC-restricted T-cell recognition. Also MHC-restriction of T-cell recognition was soon confirmed *in vivo* to be also important for antiviral protection transmissible from immune to naive recipients by immune T-cells. By 1977, A. McMichael and coworkers in I. Askonas's lab showed that human influenza virus-specific cytotoxic T-cells[51] and E. Goulmy proved that male antigen H-Y-specific cytotoxic T-cells were HLA-restricted[52].

Our results triggered heated discussions in the Department. We thought that the virus somehow altered the normal cells' MHC molecules and that this virus-specific alteration was recognized by cytotoxic T-cells in a similar way to that of foreign transplantation antigens. Everyone's imagination and intellect was stimulated to come up with a more general, simpler and more convincing explanation for the findings. Discussions were particularly lively because at the same time K. Lafferty and A. Cunningham developed their ideas on second signals necessary to induce responses against foreign transplantation antigens[53], G. Ada and R. V. Blanden where studying other virus-specific T-cell responses, L. Pilarski, P. Bretscher were thinking about B-cell responses and signal requirements along the two-signal theories of P. Bretscher and M. Cohn[54,55] and C. Parish, I. D. Gardner, I. Ramshaw, A. Happle, S. Kirov, W. Davidson, M. Dunlop and Y. Rosenberg, working on B-cell and T-cell response in various virus infections, were discussing the role of enzymes modifying carbohydrates or other self-surface structures to explain the experiments.

FURTHER ANALYSIS

We interpreted our findings to signify that virus infection somehow caused alterations of transplantation antigens on the cell surface by forming a complex of viral antigen with MHC-molecules, or undefined structural alterations or complexing of the two, and that these alterations were recognized by T-cell receptors (Fig. 1)[56]. Foreign transplantation antigens (so-called alloantigens) could then be viewed as a genetically altered form of self-transplantation antigens. This view differed from the then-favoured possibility that lymphocytes and target cells interacted mutually via transplantation antigens (see Figure), i.e. H-2^k interacted best with H-2^k, H-2^b best with H-2^b molecules in a symmetrical like-like complementarity. This intimacy model was soon excluded by the "F_1-experiment" showing that virus-specific cytotoxic T lymphocytes from heterozygote (H-2^k x H-2^b) F_1 mice consisted of at least two

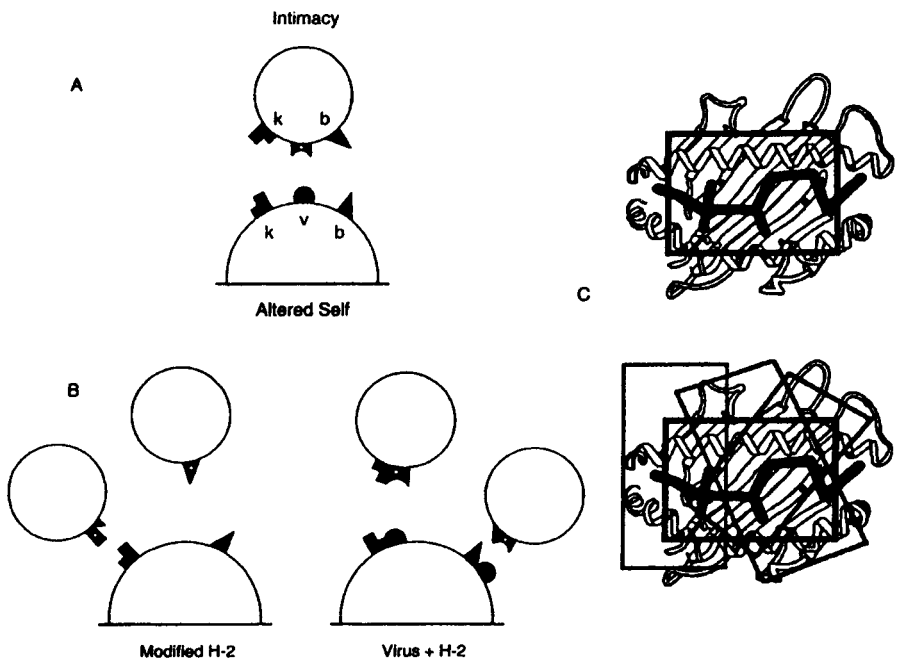

Fig. 1. *Models originally proposed to explain MHC-restricted T-cell recognition.* The idea that MHC-molecules interacted mutually to permit T-cell and target cell to come close enough together (A) was disproven by the demonstration that in an F1 between H-2^k and H-2^b (H-2^k × H-2^b) mice there were two clonally specific T-cells that were specific for virus plus H-2^k and a second population that was specific for H-2^b. Therefore T-cell receptors were either specific for the virally modified MHC molecule where neither details of either virus antigen or MHC molecules were recognized in its original form (B) or T-cells were specific for a complex formed between MHC molecule and viral antigen, that the T-cell receptor recognized parts of the viral antigen and parts of the MHC molecule. It is now clear in 1996[69, 70] that the T-cell receptor is composed of two chains Vα and Vβ that recognize a viral peptide presented by the MHC molecule[63]. What is less unclear (C), whether the T-cell receptor, here drawn as a square, interacts with the peptide plus MHC complex is always in the same directed fashion, so that certain hypervariable regions of the receptor always interact with corresponding parts of the peptide, or of the two MHC domains forming the groove (more likely), or whether alternatively the receptor interacts with the complex formed by MHC plus peptide in many possible random ways. reproduced with permission of Nature, *251*, 547, 1974, Copyright (1974) Macmillan Magazines Ltd. and JAMA, *274*, 1070, 1995.

subpopulations, one of each being specific for infected H-2^k, and the other for infected H-2^b targets. Since both MHC types were co-dominantly expressed on the lymphocyte surfaces on all host cells, this indicated that some T-cell receptors of one T-cell subpopulation were probably specific for H-2^k-plus-virus and the other subpopulation was specific for H-2^b-plus-virus.

Further experiments together with R. Blanden carried out with the help of mouse geneticists (including C. David and H. McDevitt in the U.S.A.) showed that the H-2D and H-2K regions coding for class I MHC molecules were involved in virus-specific cytotoxic T-cell recognition[46]. These findings separated the MHC-restricted recognition by virus-specific cytotoxic T-cells from the MHC class II -(H-2I)-linked immune response phenomena regulating T-cell–B-cell, or T-cell–macrophage interactions. Analyses of cytotoxic T-cell interactions *in vivo* causing lethal immunopathology[57], antiviral protection[58], and protection against *Listeria monocytogenes*[59], all confirmed that MHC restriction was also valid *in vivo.*

In our second letter to *Nature,* we therefore concluded that T-cells might function essentially by surveying the integrity of transplantation antigens. Recognition of cell surface alteration due to virus infection, chemical modification or genetic differences (i.e. alloantigens) may then be accomodated within the same model. A general hypothesis was formulated in *Lancet* in 1975[60], which stated explicitly that the function of the major histocompatibility molecules is to signal modifications of self-MHC to the immune system.

What we had discovered and tried to explain for cytotoxic T-cells, we also extended to helper T-cells, proposing that they might recognize antigen-induced modifications of Ia (as the MHC class II molecules were called at the time) on macrophages and B cells. Importantly, the results offered an explanation of the reasons for the extensive polymorphism of MHC molecules; it minimized both the possibility that some cell-destroying pathogens failed to cause immunogenic modification and the risk of there being general unresponsiveness in a population. Obviously, what was unknown at the time was that MHC molecules or transplantation antigens are the antigen-presenting molecules that are recognized as a complex with the antigenic peptide. This became known in the subsequent ten years, mostly thanks to the work of E. Unanue[61] and H. Grey and coworkers[62] for class II antigens, as well as on the particularly revealing and eye-opening work of A. Townsend[63]. He showed that class I molecules of virus-infected cells present peptides, 9–10 amino acids in length, to virus-specific cytotoxic T-cells. Similar results were obtained by J. Maryanski and coworkers[64]. These peptides became very real when they were first eluted from target cells by the group of H. G. Rammensee[65]. Peptides were also soon shown to be involved in anti-tumor CTL responses by T. Boon's group[66]. All became convincingly clear in the classical studies of P. Björkman, J. Strominger and D. Wiley in 1987, when the X-ray cristallography of a class I HLA-molecule revealed the peptide binding cleft[67,68]. It is probably not only by chance that within a few weeks of the news of this year's Nobel Prize for specificity of the cell-mediated immune response, the first studies appeared in *Science* and *Nature,* revealing the X-ray structure of the complete

complex of the T-cell receptor-MHC class I plus the bound peptide by I. A. Wilson and by D. Wiley and their coworkers[69,70]. What is less unclear, in 1996 is, which part of the TCR, and whether always corresponding parts of the TCR, recognized the peptide and the MHC molecule in the same general position[71,72] (Fig. 1).

IMPLICATIONS

The role of the thymus in MHC-restricted T-cell repertoire selection: Experiments first published by M. Bevan at MIT[73] and conducted in parallel in my lab at Scripps with the help of J. Callahan, G. Dennert and J. Klein signalled a role of thymic MHC in selection of the MHC-restricted T-cell specificity[74,75]. Reconstitution of lethally radiated H-2^b recipient mice with bone-marrow stem cells of (H-2^k x H-2^b) F_1 origin resulted in bone marrow chimeras that were tolerant of H-2^k and H-2^b; when immunised, these chimeras reacted against H-2^b plus minor histocompatibility antigens, or, in our experiments, against H-2^b plus virus only (Table 1). This indicated that MHC-restricted T-cells were specifically selected during T-cell maturation according to the MHC expressed in the thymus. This was formally shown when MHC-restricted T-cell specificity was studied in mice that lacked a thymus and therefore did not have mature T-cells. When these mice of (H-2^k x H-2^b) F_1 type were given a thymus of fetal H-2^k origin, they eventually generated effector T-cells that recognized virus-infected H-2^k, but not infected H-2^b target cells. Surprisingly, thymus deficient nude mice given a histoincompatible thymus generated T-cells that were specific for the nude mice's MHC[76], but in general the MHC (apparently of the radio-resistant part of the thymus) selected the restriction specificity of virus-specific cytotoxic T-cells.

These thymus and bone-marrow-grafting experiments had an immediate impact on clinical medicine by providing rational rules for the reconstitution of immunodeficiency disease. Accordingly, it is not only necessary to deplete T-cells in order to avoid lethal graft-versus-host disease but, in addition, the host and the transplanted bone marrow cells and the host's own or the transplanted thymus grafts must share MHC molecules. Otherwise, T-cells capable of recognizing antigen-plus-MHC molecules on infected epithelial, mesenchymal cells, macrophages or the corresponding B cells would not develop and function appropriately in such reconstituted hosts. These rules for positive selection of T-cells according to the MHC of the thymus were subsequently elegantly–and even more convincingly–confirmed with T-cell receptor transgenic mice, by H. von Boehmer and collaborators[77]. In addition several other groups also analyzed transgenic T-cell receptor expressing mice; D. Loh with an alloreactive T-cell receptor[78] and H. P. Pircher, H. Hengartner, T. Mak and K. Bürki with an LCMV-specific receptor[79]; others used a variety of additional specificities.

New vaccines: The recognition that peptides derived from viruses, bacteria, or classical parasites are presented to T-cells via MHC class I or class II molecules immediately suggested that instead of live, and therefore potentially

harmful, infectious agents, peptides could possibly be used as vaccines to induce T-cell responses[80]. This was first formally shown for virus-specific peptides in studies by M. Schulz, P. Aichele, and H. Hengartner[81], (Table 2, Fig. 2) and then by K. Melief and M. Kast and coworkers[82]. The main problem which slowed things down after the discovery of the key role of peptides by A. Townsend, was the fact that the half-life of such peptides is usually short, and protective T-cells could therefore only be induced with the help of adjuvants that guaranteed the relatively slow, long-term release of peptides, triggering T-cells over a prolonged period of time[83]. Peptide treatments were first shown to prevent EAE under various conditions[84,85]. More recently, the capacity of peptides to induce cytotoxic T-cells so exhaustively and completely that they are deleted has been shown by D. Kyburz, P. Aichele, H.P. Pircher and H. Hengartner, and D. Moskophidis[86-89]. T-cells were either induced or exhausted, depending on the relative amount and kinetics of the available antigen within a recipient mouse (Fig. 2). Thus, with too much peptide, specific T-cells could be deleted for as long as the peptide persisted–even permanently, in the case of a thymectomized host. This signalled the possibility of a "negative" vaccination-strategy (Fig. 2, Table 2): Instead of increasing T-cell precursor frequencies to enhance protection (positive vaccination), one could also reduce or delete T-cells by means of excess peptides (negative vaccination)[90]. The latter possibility may allow the immunopathological, disease-causing T-cells to be exhausted and deleted. Although one such example has been documented in an MHC class I-specific immunopathological T-cell-mediated transgenic diabetes model[87], attempts to achieve the same in already primed hosts, before or after initiation of disease, have only met with partial success.

T-cell epitope escape mutant viruses: It could be expected that noncytopathic vi-

Table 2. The role of antigen localisation, dose and time on "positive" and "negative" vaccination.

vaccination	**Infection with**				
	cytopathic viruses		noncytopathic viruses		
	localized	generalized	localized		generalized
			choriomeningitis	subcutaneous	
none	survival	death	death	disease	disease but survive (virus eliminated)
positive vaccination	protection	protection	protection (virus eliminated)	disease (shorter)	death or protection (virus eliminated)
negative vaccination by "exhaustion"	disease and death dependent upon critical role of cytotoxic T-cells		Treated mice do not develop disease and survive but do not eliminate virus (develop carrier status).		

Summarized from P. Aichele, 1994[87]; M. Schultz, 1991[81]; St. Oehen[88], P. Aichele, 1995[88a], P. Aichele, 1990[114].

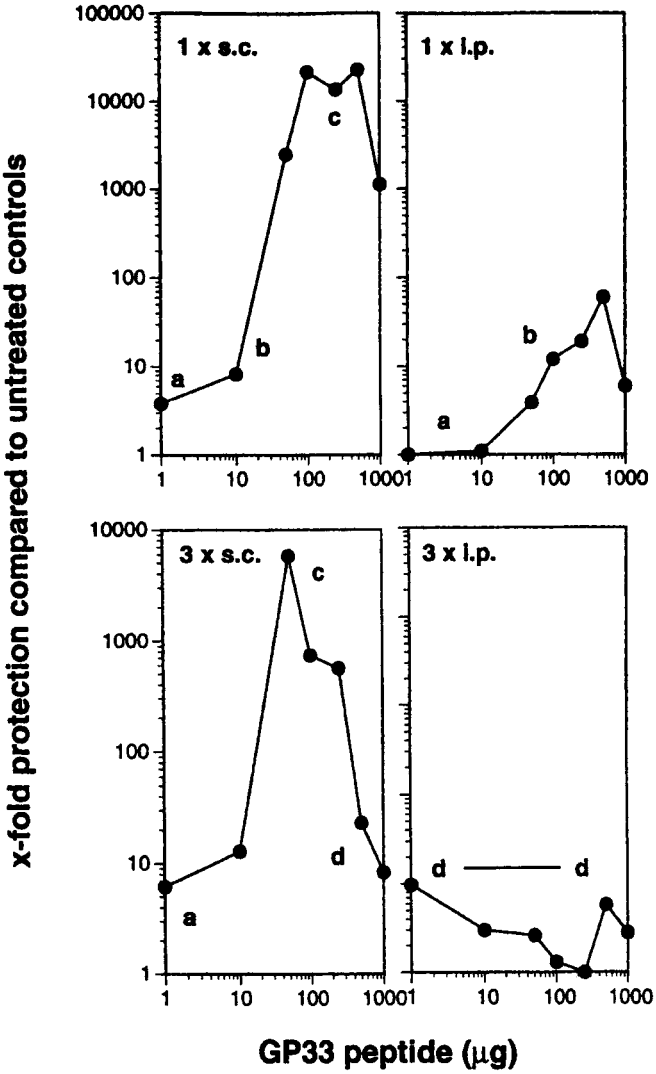

Fig. 2. *The role of antigen localisation and of dose and time on T-cell responses:* The LCMV-glycoprotein peptide GP_{33-41} presented by MHC class I D^b in C57 BL/6 H-2^b mice was used as vaccine. Its half-life *in vivo* is <<12 hours; the read-out chosen in this experiment to assess T cytotoxic specific, LCMV specific cytotoxic T-cell immunity is the fold protection compared with controls; protection was titrated against amount of peptide with adjuvans given either 1 time or 3 times subcutaneously (s.c.) or 1 time or 3 times intraperitoneally (i.p.). No induction was seen if antigen is available in too small amounts (a), or for too short a period of time (b). Induction of a protective immune T-cell response occurs if sufficient antigen is available for long enough (c). In contrast, too much, excessive, induction of all inducible T-cells by too much antigen spread throughout the organism and available for too long causes exhaustion/deletion of all specific T-cells (d). Modified from Aichele et al., 1995[88a].

ruses would mutate the 9-10 amino acid peptides recognized by T-cells in the context of the crucial MHC class I antigen. Mutation of this peptide, so that either its presentation by MHC molecules or its recognition by T-cells is no longer possible, could help viruses to escape immune surveillance. A first example of this possibility was found by chance when H. P. Pircher, D. Mosko-

phidis and H. Hengartner analyzed T-cell receptor transgenic mice that expressed a T-cell receptor specific for the LCMV glycoprotein peptide 33-41 presented by the MHC class I (D^b) molecule[91,92] (Fig. 3). When we infected such mice in the footpad we found a very early swelling reaction caused by immunopathological cytotoxic T-cell response by day 2 to 4, which, however, waned, and a second CTL-mediated response was measurable after day 8 (Fig. 3). When this unexpected double peak of the T-cell-mediated footpad swelling reaction was analyzed further, it became clear that the virus had mutated by day 6 of infection; it no longer expressed the original gp 34-41, but exhibited various mutations within this epitope, presented by the MHC class I (D^b) molecule[91]. Apparently the vehement virtually monoclonal antiviral $CD8^+$ T-cell response in the T-cell-receptor-transgenic mouse had quickly selected the T-cell-epitope-mutant virus that had escaped the transgenic T-cells. A similar mutant virus that escaped the T-cell response has subsequently also been found in patients infected with HIV by R. Phillips, A. McMichael and coworkers[93] and in HBV-infected patients by Bertoletti *et al.*[94].

MHC-disease associations: The linkage between some disease susceptibilities and certain HLA-types was one of the first findings signalling the important role of MHC-molecules in immunity (reviewed in[95,96]). These diseases are often of autoimmune or immunopathological nature and are often linked to HLA class I, rather than class II, molecules[97]. The critical role of modified MHC molecules in T-cell recognition explained why different allelic forms of

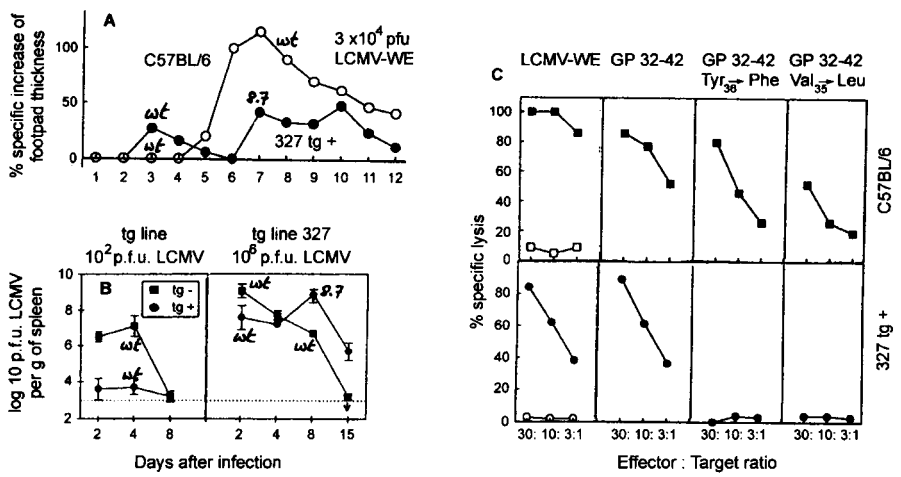

Fig. 3. *Selection of cytotoxic-T-cell-epitope escape mutants in T-cell receptors of transgenic (TCR-tg327) mice.* LCMV-WE wild-type (wt) was injected into the footpad (A). The control. C57BL/6 developed the expected $CD8^+$ T-cell-dependent footpad swelling by day 6–7, the TCR-tg327 mice exhibited an early swelling caused by the tg-TCR specific for LCMV-GP_{33-41} plus D^b. This early response selected a mutant virus 8.7 by about day 7–8, that induced a swelling reaction by the endogenous non-tg T-cells specific for other peptides. The virus titers found in the spleen of mice infected with a low (10^2 pfu) or a high (10^6 pfu) dose of LCMV-WE is shown in (B). All mice were infected with LCMV wt; a variant virus 8.7 was selected in tg line327 mice infected with 10^6 pfu of LCMV wt by day 8 (B). The cytotoxic T-cell activity found in control C57BL/6 or TCR-tg327 mice on day 8 is shown in C. The peptides of the T-cell-epitope escape variant virus are not recognized by the tg-TCR, but are still partially (Tyr →Phe) or not (Val→Leu) recognized by effector T-cells from control C57BL/6 mice. Summarized from Pircher et al., 1990[91].

MHC are randomly distributed in the population and made it likely that infectious agents and their peptides are presented by at least one of the four to ten MHC molecules expressed by an individual; this minimizes the possibility that a virus escapes immune surveillance, endangering survival of the entire population. The fact that some differences with respect to antigenicity and immunogenicity are linked to the MHC, and correlate directly with different strengths of the T-cell responses, shows that different MHC molecules directly determine and regulate resistance to diseases.

Obviously, cytopathic viruses must be controlled efficiently by the immune system, otherwise the host species dies. Therefore, hosts with non-responder or non-presenting MHC-molecules have probably been eliminated long ago by natural selection, leaving only high responders to survive. In contrast, for noncytopathic viruses, diseases are not caused by the infectious agent itself, but rather by the damaging effect of protective T-cell responses. Because such agents do not directly cause disease, they do not exert a direct selective pressure on survival. But since these viruses may induce immunopathological cytotoxic T-cell responses, differences in MHC may influence the severity of the disease. In fact, many of the diseases exhibiting some association with MHC have an aura of being autoimmune or immunopathologically mediated. This has been directly shown for known noncytopathic viruses[97,98] (Table 3).

Table 3. MHC-disease association reflecting CD8⁺ T-cells mediated immunopathology against host cells infected with noncytopathic LCMV.

Mouse strain	A. Maximal increase of serum transaminases after i.v. infection with LCMV (pfu): T-cell mediated hepatitis				B. Mortality after intracerebral infection with LCMV	
	WE_{UBC} A		WE_{UBC} D		WE_{UBC} A	WE_{UBC} D
	10^2	10^6	10^2	10^6		
BI0.G H-2D^{q}L^q	–	–	–	++	100 %	100 %
BI0.BR H-2D^k	–	–	++	±	100 %	0 %

Mice were infected i.v. with the indicated doses of LCMV WE_{UBC} A or D. Maximal increases of serum transaminases were indicated with –: < 3 fold, +: 10-20 fold, ++: > 100 fold. Damage of liver cells or of choriomeningeal or ependymal cells caused by LCMV-specific CD8⁺ T-cells. In absence of CD8⁺ T- no immunopathology is seen.

C. Role of virus characteristics increasing likelihood to establish persistent LCMV-infection in mice

Virus parameters:	Increased replication rate, increased resistence to interferons, loss of T-cell epitopes by mutation, increased tropism for lymphohemopoietic cells.
Host parameters:	Decrease in relative interferon levels, loss of presenting MHC class I molecules (e.g BALB/c dm2 mice), loss of CD8⁺ T-cells (e.g. DBA/2 mice), loss of T-help, lack of IL-2.

Summarized from R. M. Zinkernagel, 1985[98]; T. P. Leist, 1989[100]; D. Moskophidis, 1994[101].

As pointed out above, one of the motivations for testing various mouse strains for cytotoxic T-cell activity, resulting in the discovery of MHC-restricted T-cell recognition, was the variable and weak evidence of susceptibility differences of mice to lethal choriomeningitis that somehow correlated with MHC (Table 4). The comparison of a slowly replicating neurotropic strain of LCMV (UBC-A), with a rapidly replicating viscerotropic isolate UBC-D characterized by Ch. Pfau[99], revealed a dramatic and strict correlation with MHC when the different diseases were studied. After intracerebral infection of mice, UBC-A virus caused death in all recipients, irrespective of MHC. UBC-D caused immunopathological disease in all those possessing the MHC class I H-2D^{q}L^{q} allele but not in the others[98,100]. Furthermore, the susceptibility of mice to becoming persistently infected virus-carriers could be linked by D. Moskophidis to the absence of class I MHC molecules that were able to present the critically important dominant immunogenic viral peptide[101,102]. These studies, in addition, demonstrated that virus dose and virus strain (including T-cell epitope variants) also played an important role in the overall virus-host balance and in MHC-disease association.

Table 4. Transmission of protective immunity from mother to offspring.

Because of MHC-restriction of T-cell recognition, T-cell maturation in offspring is slow and starts after birth to prevent graft-versus host disease. During this period of physiological immuno incompetence transfer of maternal antibody-memory is essential for protection of offspring against many infections.

	preexistent antibodies	"activated" T-cells
placenta	maternal antibodies transmissible except with chorio-epithelial double layered placenta (bovines)*	non-transmissible, danger of host-versus-graft disease and rejection
milk	transmissible via gut of offspring (temporarly) passive protection in gut*	evidence poorly analyzed
function	*"altruistic":* protection of *offspring* against infectious diseases during the period of T-cell immunodeficiency and protect mothers during pregnancy	*egoistic:* protection of *original host* against spread of non-cytopathic infectious agents (controls immunopathology and tumor cells within host)

*Summarized in Brambell[106a].

Thus MHC (in humans HLA-)–disease association may not be found readily for acute cytopathic viral or bacterial infections, because natural selection has favoured good MHC-presenter-virus combinations[90]. In contrast, for non-cytopathic viruses, the selection pressures are much weaker and various virus-host immune response balances resulting in more or less immunopathological disease are acceptable for species survival. Because cytotoxic and protective T-cell responses directly depend upon class I MHC presentation of peptides, susceptibility to consequential disease may correlate directly with the MHC class I allele, depending upon the localisation of infection (choriomeningitis vs. establishment of a virus carrier status)[98,101].

One may therefore extrapolate that at least some autoimmune diseases may eventually turn out to be caused by immunopathological T-cell responses against viruses that are poorly cytopathic or noncytopathic[95, 96] (Fig. 3, 4). Hepatitis B, C, or D virus infections or possibly immunodeficiency virus (HIV) infections in man may represent this type of chronic infection leading to a protective but immunopathological T-cell response, causing MHC-regulated immunopathologic disease. Alternatively, infectious agents that are either unknown or not yet recognized may be involved initially in triggering the disease by immunopathological or autoimmune mechanisms. For example, it is suspected that also common cytopathic viruses may be involved in the pathogenesis of some autoimmunity diseases (Fig. 4, 5). Infections of MHC class-I-positive epithelial or neuroendocrine cells should be controlled primarily via $CD8^+$ T-cells; their efficiency will, therefore, not only determine the extent and kinetics of destruction of infected host cells, but also whether an autoimmune T-cell and B cell response against such sequestered self-antigens is induced.

The reason for MHC class I linkage of autoimmune diseases is, therefore, probably in some diseases that the autoimmune effector T-cells are class I restricted (Fig. 4), and in other situations that modulations of common or unrecognized infections by the efficiency of class-I-restricted effector T-cells may indirectly regulate autoantibody responses (Fig. 5).

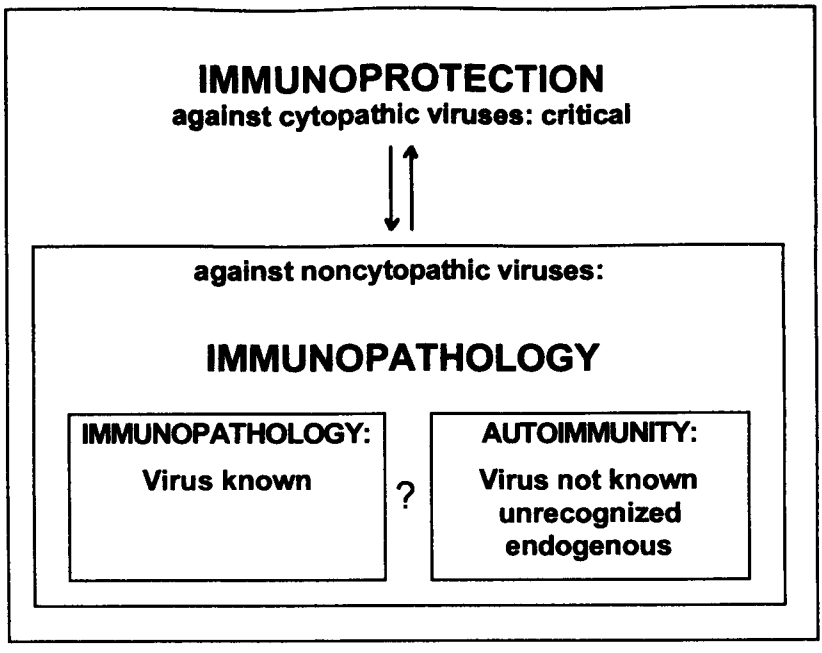

Fig. 4. *Immunoprotection and immunopathology:* Efficient immunoprotection is mandatory for survival of the host infected with a cytopathic virus. Since noncytopathic viruses do not cause cell damage directly, pathology is caused by immune mechanisms. If the virus is not recognized or is unknown, the disease may be mistaken as an autoimmune disease.

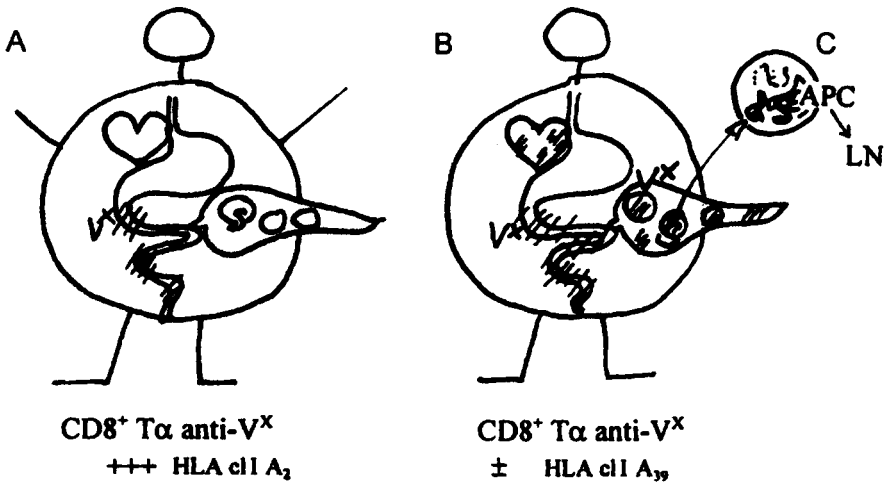

Fig. 5. *Autoimmunity induced by viruses.* If a cytopathic or noncytopathic virus is stopped early enough by MHC-class I-(HLA-cl I A_2)-restricted effector T-cells (A), sensitive target cells are not destroyed. If the protective HLA-cl I A_{39}-restricted T-cell response is slow (B), virus may reach sensitive target organs and cells that may be destroyed directly or induce disease by T-cell-mediated immunopathology, i.e. cause release of self (S)-antigen. The self-antigen may then be picked up and presented by APC (C) and in local lymph node or in the spleen induce autoimmune T-cells and/or autoantibodies. Autoantibody production needs T-help that may be provided by newly induced $CD4^+$ T-cells specific either for viral antigens that are linked to self-antigens or are specific for so far segregated immunologically ignored self-antigens.

Consequences for immunological memory: The discovery that T-cell specificity monitored MHC molecules, that it was positively selected in the thymus, and that this explained the transplantation reaction, offered a basis for understanding the role of protective immunological memory. Immunological memory by B and T-cells is an important hallmark of immunity and has been exploited successfully by vaccinations (reviewed in[103-106]). However, before vaccinations were used, the idea of immunological memory had two raisons d'être. One could argue that if a host dies during the primary infection, he does not need an immunological memory. Also, if he survives the primary infection, he does not need memory because the system has proven itself efficient. What then is the function of immunological memory? The first and most critical one is via transfer of immune antibodies of the mother to protect offspring during the phase of maturation of the immune system after birth, the second is to protect mothers from infections during pregnancy (reviewed in[106a, b]). I will only discuss the first aspect further here.

Calves are born without antibodies because, as for all vertebrates, the immune system is not yet mature enough to produce its own antibody response[106a] (Table 4). In addition, in calves, maternal antibodies cannot be transmitted because of the complete doubly layered placenta. All protective antibodies are transmitted via colostral milk from the mother within 24 hours of birth. If this does not happen, the calf dies within a few weeks as a result of common bacterial infections.

Why then, are newborn vertebrates immunoincompetent? (Table 4). My explanation is, that because of MHC-restricted T-cell recognition, T-cells of

the fetus should not mature, so as not to endanger pregnancy, or cause graft-versus-host disease against maternal MHC molecules[90,106]. The passively acquired antibodies provide protection during the critical period after birth, during which T-cell and B-cell maturation slowly takes place over 3–6 months in humans, or four weeks in mice. Since maternal antibodies must cover a wide spectrum of relevant infectious agents that may not be encountered during pregnancy, immunological memory carried by antibodies is mandatory for at least two reasons. Firstly, pregnancy is relatively short compared to the time taken for the host to reach sexual maturity, and therefore antibody memory must be developed over a long period. Secondly, the relevant infections must not occur during pregnancy, because many infections cause damage to and abortion of the foetus.

Evidence has been found in various models and infections that persistence of antigens both, in antibody-antigen complexes and exposed on follicular dendritic cells, maintains antibody responses for many (Table 5) years, to guarantee levels of transmissible protection[107–109].

Table 5. "Memory B-cells" are not protective whereas preexistent neutralizing levels of antibodies protect naive recipients against cytopathic infectious agents.

Adoptive transfer to naive mice	Booster immunization 2 d before challenge infections	Result of challenge infection
B-cells, naive or normal	no	disease
	yes	disease
"primed" or "memory" B-cells	no	disease
	yes	disease
immune serum with neutralizing antibodies	no	protection
	yes	protection

Summarized from experiments performed in mice with vesicular stomatitis virus (VSV) in refs.: U. Steinhoff, 1995[108]; M. F. Bachmann, 1994[115].

What then is the role of T-cell memory?

Memory T-cells are probably necessary to maintain memory B cells but this is not yet certain. Although increased precursor frequencies of T-cells provide some protection, which should suffice to protect mothers during pregnancy, there is a second very important aspect of T-cell memory. T-cells are critical for controlling persisting noncytopathic viruses that hide in peripheral epithelial or mesenchymal cells and prevent them from reemerging to trigger T-cell mediated immunopathological disease (Table 4). There is good evidence available now that these protective T-cells are also maintained by antigens[106,110,111], although this simple notion is very controversial at the present time[102,112,113]. Thus, low levels of ongoing T-cell responses reflect antigen-driven activated effector T-cells and not special memory T-cells. These serve to protect the host itself and not progeny; these *egoistic* protective memory T-cells cannot be transmitted, due to MHC-differences between mother and offspring (Table 4). Taken together, the summarized evidence suggests that because of MHC-restricted T-cell recognition which causes the trans-

plantation reaction, prolonged physiological immunodeficiency of the offspring is necessary; protection during the period of maturation of the immune system is provided by passively transferred *altruistic* antibodies, which necessitate a mandatory antigen-driven elevated antibody level in mothers (Table 4).

CONCLUSIONS

In the complex balance between viruses and hosts T-cells play an important role that varies with different host and viral characteristics. The unexpected finding, by chance and necessity, of MHC-restricted T-cell recognition, in a field of biological science prepared by immunologists, geneticists and virologists, has triggered a great number of subsequent important studies by many other groups, resulting in an excellent molecular understanding of T-cell recognition of virally infected target cells. These combined findings have helped to understand better both immunological specificity and immunological memory. They have furthered our understanding of disease pathogenesis and made possible applications of the acquired knowledge for improving protective immunity and for diminishing immunopathological T-cell responses.

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Erratum

Page 4, line 23 should read as:

responses towards invading microorganisms or against cancer metastases. Our