The discovery of MHC restriction

Rolf M. Zinkernagel and Peter C. Doherty

The chance discovery 20 years ago that virus-specific cytotoxic T lymphocytes recognized antigen together with major histocompatibility complex (MHC) class I glycoproteins was the key to understanding immune surveillance of self. The altered-self hypothesis that was developed to explain these findings provided a reasonable biological basis for alloreactivity, MHC polymorphism and immune response (Ir) gene effects. Here, Nobel Laureates Rolf Zinkernagel and Peter Doherty remember the story behind the discovery of MHC restriction[*].

The understanding of the major histocompatibility complex (MHC) in 1973–74 reflected several different, complementary themes, some of which had been pursued for more than 30 years. The murine transplantation antigens (H-2) had been defined by Gorer[1] and Snell[2], based on the early work of Little, Strong and others (particularly at Bar Harbor), who developed inbred strains of mice in order to transplant tumors (reviewed in Ref. 3). Over the subsequent years, the dissection of graft rejection led to the development of a range of H-2 recombinant and mutant mice that later proved invaluable for the rapid definition of MHC-restricted T-cell responses. Hematologists, particularly Dausset and van Rood, had used serological approaches to define the human lymphocyte antigen (HLA) system[4,5]. As more and more patients were tested through the late 1950s and early 1960s, it became apparent that susceptibility to some diseases was linked to HLA phenotype (reviewed in Ref. 6). Subsequent detailed studies of antibody and delayed-type hypersensitivity responses by Benacerraf[7] and McDevitt[8] and colleagues, and susceptibility to tumors by Lilly[9], showed MHC-related or linked differences for inbred mice and guinea-pigs, respectively.

The missing piece of the puzzle was that there was no clear idea what the strong transplantation antigens were for: surely the system had not developed just to frustrate transplantation surgeons. Speculations were that these highly polymorphic glycoproteins had evolved to prevent mutual parasitism or tumor cell transmission[10], or to cause rejection of mutant thymocytes[11]. Others argued that the extreme variation in MHC phenotype might stop viruses (or other pathogens) from mimicking all transplantation antigens and thus eliminating the species, or that they functioned as enzymes or as generators of antibody specificity[12,13]. In hindsight, the most perceptive guess was that of Lawrence[14], who suggested in 1959 that infectious agents complexed with transplantation antigens (self + x) intracellularly and triggered lymphocytes to produce a soluble, specific receptor for this complex (transfer factor); we only became aware of this hypothesis after we both moved to the USA in 1975. Accounts of the ideas and technology that were current immediately prior to the finding of MHC restriction are given in the first edition of Klein's magnificent book *Biology of the Mouse Histocompatibility-2 Complex*[3], and in a review by Katz and Benacerraf[15], which were both published in 1975.

The investigators, the experiment and the environment

How were two very junior investigators working at the John Curtin School of Medical Research (JCSMR) in Canberra, Australia, able to trigger a major paradigm shift in immunology? What happened reflected the local scientific environment, our own scientific upbringing, sufficient ignorance to be able to look at new findings from first principles and enormous personal efforts during a period of intense collaboration, which lasted only about two years. In order to convince young people that absolutely anybody can achieve some measure of success in science, we provide the following personal details.

Rolf graduated from Basel University Medical School and thought of becoming a neurologist or a surgeon. He spent a few months at the Salpétrière in Paris and then, after having obtained his MD, worked as an assistant at a local surgical clinic in Basel. Both he, and his chief, rapidly came to the view that his real talent had to lie elsewhere. Fortunately, the University of Zurich offered a post-MD course in Experimental Medicine, the aim being to strengthen Swiss clinical research. His commitment to immunology was triggered, in particular, by J. Lindenmann. The next two years were spent in the laboratory of H. Isliker in Lausanne. His project, influenced by the seminal studies of Brunner and Cerottini[16,17], was to establish an assay for antibody/complement-mediated lysis of ^{51}Cr-labeled enteropathogenic *Escherichia coli*. The test never worked. Involvement in bacterial pathogenesis and immunity, however, made him very aware of the experiments of G.B. Mackaness, R.V. Blanden and G.L. Ada with the bacterial models. A fellowship from the Stiftung für Biologisch-Medizinische Grundlagenforschung funded him to go to Canberra to work with Bob Blanden. He arrived in January 1973 and started to work on immunity to *Listeria*.

Peter trained in Veterinary Science at the University of Queensland. Interest in immunology was stimulated by lectures from the elder J. Sprent (Professor of Parasitology), reading F.M. Burnet's books on virology and immunology, and papers on viral pathogenesis and immunity by C. Mims at the JCSMR. His university fees were paid by the Agriculture Department, requiring him to spend four years in the state veterinary laboratory in Brisbane. Much of this time was spent doing research on bovine leptospirosis and starting out in virology. A move to the Northern Hemisphere took him to the Moredun Institute, where he became an

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experimental neuropathologist and completed a PhD (Edinburgh University) on louping-ill (a tick-borne flavivirus) encephalomyelitis. The most interesting aspect of this study (with Hugh Reid) was the demonstration of virus-specific antibody production in the central nervous system. He returned to Australia from Scotland to work with C. Mims in December 1971. C. Mims moved to London in mid-1972, leaving Peter his technician (Gail Essery) and the lymphocytic choriomeningitis virus (LCMV) model, a legacy from F. Lehmann-Grube who spent two years in Canberra in the early 1960s. Peter attacked the immunopathology aspect of LCM, exploiting a technique for obtaining mouse cerebrospinal fluid (CSF) to quantitate viral meningitis learned from a chance encounter with R. Carp[18]. When Rolf arrived in Canberra, he was put into the laboratory with Peter and Gail. Rolf collaborated with Bob on experiments with the bacterial models, while together we started to explore the role of cytotoxic T lymphocytes (CTLs) in the lethal choriomeningitis triggered by LCMV.

All the initial work on antiviral CTLs was done with LCMV by Oldstone[19], Cole[20], and Marker and Volkert[21]. They found that the ^{51}Cr-release assay developed by Brunner and Cerottini[16,17] to study graft rejection could be used to measure CTL activity in LCMV infection. The assay was brought to Canberra by Bob who, with his graduate student Ian Gardner, analyzed the CTL response in mice infected with ectromelia (mouse pox) virus[22]. We decided to use the LCMV CTL assay to see if the inflammatory cells that we recovered from the CSF of mice with clinical LCM were cytolytic *in vitro*. Because we had only small numbers of cells to work with, we miniaturized the ^{51}Cr-release assay by adapting it to 96-well plates. These experiments were successful and revealed potent antiviral CTLs, suggesting that T-cell-mediated destruction of LCMV-infected meningeal and ependymal cells *in vivo* was the essential pathogenetic mechanism[23]. We postulated that acute brain edema, resulting from CTL-mediated damage to the blood–brain barrier[24], caused death by compression of the brain stem. When the vital dye Evans' blue (after Paul Ehrlich!) was injected intravenously, the brains of LCMV-infected mice that had effector CTLs, but not of T-cell-depleted controls, turned blue[24].

In March 1973, a paper appeared by Oldstone, McDevitt and collaborators, indicating that mice of different MHC (H-2) types exhibited distinct lethality patterns and kinetics of disease after intracerebral LCMV infection[25]. This stimulated us to ask whether the notion that antiviral CTLs were responsible for the fatal choriomeningitis could be tested further by correlating the severity of the clinical disease in mice of different H-2 haplotypes with the level of lytic T-cell activity. Some 6–8 mice of each of the inbred and cross-bred strains available at the JCSMR were challenged with LCMV. Two of each were sampled on day 7 after infection, when mice normally become sick, to test for CTL effectors in spleens. The remainder were monitored until time to onset of lethal disease. The first experiment in late August 1973 gave a clear result that did not fit our predictions. Only some of the mice seemed to be generating virus-specific CTLs, although all succumbed to LCMV, some on day 7, some a few days later, and all by day 11 or 12. Either the level of CTL activity had nothing to do with the induction of lethal choriomeningitis, or our CTL assay system was missing something. It quickly became obvious that the latter was the case.

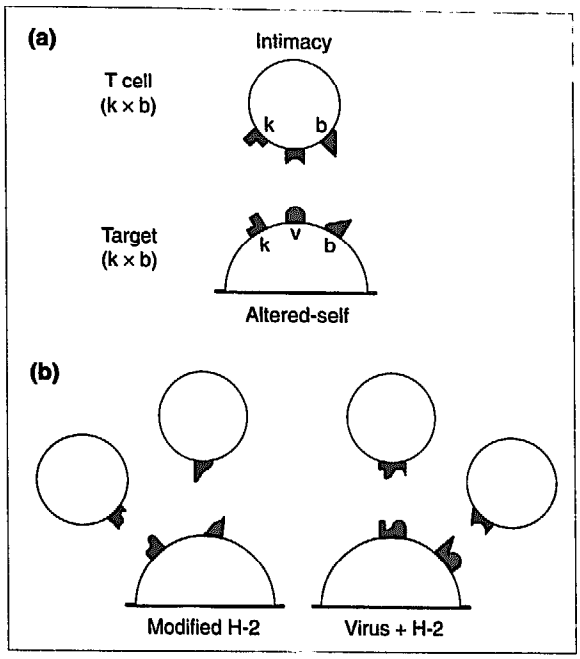

Fig. 1. *Models describing the capacity of F_1 H-$2^{k/b}$ T cells to interact only with histocompatible virus-infected target cells (modified figure reproduced with permission from Ref. 39). (a) The intimacy concept proposes a single immunologically specific T-cell receptor (TCR) for viral (v) antigen, which is additional to a requirement for physiological interaction coded for by the H-2 gene complex (mutuality between either H-2^k or H-2^b). (b) The altered-self concept postulates that there are at least two T-cell populations with receptors of different immunological specificities recognizing modified H-2 or virus plus H-2 of either parent type.*

We were in a Department of Microbiology, dominated by virologists. Plaquing of virus on tissue culture cells was a standard procedure, and a central facility provided single-cell suspensions of monkey (Vero), hamster (BHK) and mouse (L929) cells twice weekly. We all used L929 cells for CTL assays because they were of murine origin and were readily infected with both LCMV and ectromelia. By chance, the mouse strain available in greatest numbers was the CBA/H strain. The L929 cells had been derived from C3H/He mice, which share the H-2^k haplotype of the CBA/H. All the LCMV-immune spleen cells from H-2^k mice, including F_1s, lysed infected L929 cells. By contrast, spleen cells from mice that were H-2-different, namely BALB/c (H-2^d) and C57BL/6 (H-2^b), failed to do so. This was surprising, since earlier experiments at The Johns Hopkins University, using allogeneic combinations of immune T cells and infected targets, had shown what was believed to be LCMV-specific CTL activity[20].

We duplicated our basic findings in two experiments over the subsequent weeks. However, it was obviously essential to show that LCMV-immune lymphocytes from mice that did not express H-2^k were indeed able to lyse LCMV-infected, H-2-compatible target cells. This proved to be more difficult than expected, because the

other mouse cell lines available in the department (H-2^d mastocytoma P815, or the H-2^b thymoma EL4) could not be infected with LCMV and mouse embryo fibroblasts proved to be very 'leaky' for the ^{51}Cr label. We thus asked whether cells isolated from the peritoneal cavity of mice could be used as a primary source of target cells, a strategy suggested from Rolf's work with *Listeria*, which (as first shown by Mackaness[26]) grows well in macrophages. The plastic-adherent cells from peritoneal exudates were readily infected and were labeled with ^{51}Cr. In October 1973, criss-cross experiments showed that LCMV-immune T cells from H-2^b mice lysed LCMV-infected macrophages of H-2^b, but not other H-2 types, and vice versa. The initial results and speculations were summarized at the end of an account of the LCM immunopathogenesis studies that we had been writing for *Transplantation Reviews*[24], and a detailed report was submitted (via John Humphrey) in early December for publication as a letter to *Nature*. It was accepted in January and appeared in April 1974 (Ref. 27).

The first public presentations of this work outside Australia were at a meeting at Brook Lodge (MI, USA) attended by G. Ada, and at the Keystone meeting in Squaw Valley (CO, USA) attended by A. Cunningham, in February and March 1974, respectively. A letter sent back to Canberra summarized data by Gene Shearer showing that trinitrophenyl (TNP)-specific CTLs lysed syngeneic TNP-modified targets better than comparable allogeneic targets; Gene submitted this to the *European Journal of Immunology*[28] shortly after our report in *Nature* appeared. Obviously, the two sets of findings were made completely independently.

There were other observations already in the literature that were relevant to our initial findings. Lévy[29] and Herberman[30] and colleagues had published data indicating preferential lysis of H-2-compatible targets by leukemia-virus-specific CTLs. Kindred and Shreffler found that H-2-incompatible T helper cells transfusing to nu/nu mice were unable to provide help for nu/nu B cells[31]. McCullagh[32], and Katz, Hamaoka and Benacerraf[33] had shown separately that histoincompatible B cells, when mixed with T cells and antigen *in vitro* or *in vivo*, generated antibodies without a need for specific T-cell help. This 'allogeneic effect' suggested that reaction against foreign transplantation antigens expressed on the B cells could substitute for conventional T-cell help. Katz and Benacerraf also confirmed Kindred and Shreffler's finding that MHC-matching optimizes T-cell help. However, the experimental systems were complex, and did not make development of simplifying models an easy matter[15]. Using inbred strains of guinea-pigs in a more direct experimental system, Shevach and Rosenthal[34,35] found a tenfold enhancement of antigen-specific proliferative T-cell responses if the primed T cells and antigen-pulsed macrophages shared responder-MHC types. We were lucky that our virus model, and our relative freedom from much of the preceding debate, allowed us to develop a (naive) simplifying model.

The discussion and the interpretation

We thought from the outset that we had discovered the key biological role for strong transplantation antigens and, as we are both rather noisy and the claim was not very modest, our results stirred up a tremendous amount of discussion among the immunologists at the JCSMR. There was a continuing debate in Ada's Department of Microbiology, which helped greatly in the clarification of intellectually satisfying hypotheses. In addition, the findings on MHC restriction shared the limelight with Lafferty and Cunningham's ideas on second signals (factors) necessary to induce responses against foreign transplantation antigens[36] (reviewed in Ref. 37). New and interesting data were constantly emerging from the laboratories of Bob Blanden (cell-mediated immunity), A. Cunningham, L. Pilarski and P. Bretscher (studying B cells and antibody specificities for heterologous red blood cells and looking for B cells with sombrero plaques for donkeys and sheep[38]), and theoretical immunologists who thought about general rules and asked why T cells should kill. C. Parish and W. Davidson were establishing cell-separation techniques that have been widely used over the years. Ian Gardner and Bob Blanden rapidly confirmed the MHC-restriction finding for ectromelia-virus-specific CTLs. I. Ramshaw, A. Hapel, S. Kirov, M. Dunlop and Y. Rosenberg were studying B- and T-cell responses in various models. Lafferty's input was unforgettable, especially when the animal caretaker (wanting a quiet Christmas) let us use all his mice over the long summer vacation. The late, lamented and unforgettable Bede Morris (Professor of Immunology) maintained a strong position of skepticism, while his colleague, P. McCullagh, provided considerable perceptive input from his own studies on tolerance.

We proposed alternative possibilities to explain the restriction of effector T-cell function by the strong transplantation antigens (Fig. 1)[27,39]. The first was the mutual recognition (two receptor) idea that had been raised by others (reviewed in Ref. 15). The alternative was that a single T-cell receptor (TCR) was recognizing 'altered-self' MHC antigen, either as a complex formation between viral and MHC molecules, or due to some virus-induced change in the MHC molecules. The idea that viruses modify self had been around for some time (reviewed in Ref. 24), and was a fairly obvious conclusion for anyone who had been working in virology. However, the possibility that self was defined by the H-2 molecules had not been discussed in this context.

The then favored possibility was that lymphocytes and target cells interacted mutually via transplantation antigens; that is, that H-2^k recognized H-2^k in a like–like fashion[15,35,39]. This mutual interaction or intimacy model was soon excluded by experiments showing that virus-specific CTLs from heterozygous (H-2^k × H-2^b) F_1 mice comprised at least two subpopulations, specific for either LCMV-infected H-2^k or H-2^b targets. Since MHC molecules are codominantly expressed on lymphocyte surfaces, H-2-restricted recognition signaled TCR specificity rather than like–like interactions.

The first experiment that mapped the effect to the class I regions of the MHC was carried out using the A/J H-2-recombinant A/J (H-$2K^kD^d$) mouse strain, which we obtained from the Zoology Department in the University[40]. Definitive evidence that the H-2K and H-2D (not H-2I) loci provided the CTL MHC restriction elements for both LCM and ectromelia was generated with a range of H-2-recombinant mice that Bob Blanden obtained from D. Shreffler

and C. David at Washington University, St Louis. This was published in an alphabetically authored letter to *Nature* in early 1975 (Ref. 41). Experiments demonstrating that MHC class I restriction also applied to effector T-cell function *in vivo* were quickly done with the LCMV immunopathology model[42] to show antiviral protection[43], to provide evidence that heterozygote advantage could be a factor in the maintenance of MHC polymorphism[44] and to reveal that immunity to intercellular bacteria was also MHC restricted[45].

The ideas that we developed concerning the physiological function of the MHC were published in the 'hypothesis' format of *The Lancet* in the summer of 1975 (Ref. 46). The article, entitled 'A biological role for the major histocompatibility antigens', discussed class I and class II MHC restriction, and proposed a unifying view that helper and cytotoxic T cells were specific for the appropriate 'altered-self' MHC glycoproteins. We argued that surveillance of self was essentially analogous to alloreactivity, that levels of T-cell responsiveness could reflect the formation of an appropriate 'altered-self' and that the extreme polymorphism of the class I molecules could be explained both by differential responsiveness and by heterozygote advantage[46]. A new beginning had been made in the biological definition of the mechanisms underlying T-cell targeting and self–nonself discrimination in immunity. The molecular basis of these events was to emerge from other laboratories over the next 10–30 years.

We owe a great debt of thanks to Kathrin and Penny, who juggled work, small children and two obsessed maniacs through this period of intense activity, our colleagues in Canberra who provided the necessary intellectual tension and forced us to justify our thinking in a very critical milieu, and the taxpayers of Switzerland and Australia who footed the bill. We are also grateful to the general scientific culture in Australia, which supported virologists and immunologists who established the basis of resources that enabled our work to be done in sufficient isolation to allow the quiet development of something novel.

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