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 we developed to explain these findings provided a reasonable biological basis for alloreactivity, MHC polymorphism and immune response to gene effects. Here, Nobel Laureates Rolf Zinkernagel and Peter Doherty remember the story behind the discovery of MHC restriction.

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 Basle University Medical School and thought of becoming a neurologist or a surgeon. He spent a few months at the Salpêtrière in Paris and then, after having obtained his MD, worked as an assistant at a local surgical clinic in Basle. 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. His next two years were spent in the laboratory of H. Islikcr in Lausanne. His project, influenced by the seminal studies of Brunner and Cerottini[19,20], was to establish an assay for antibody/complement-mediated lysis of 51Cr-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 assistant to the Neurologist, Peter Doherty was commissioned by Howard Goodman.

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[21] and Snell[22], 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.[23] 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. 4). Subsequent detailed studies of antibody and delayed-type hypersensitivity responses by Benacerraf[24] and McDevitt[25] and colleagues, and susceptibility to tumors by Lilly[26], showed MHC-linked or linked differences for apparent that susceptibility to some diseases was linked to HLA or to cause rejection of mutant thymocytes.' We are grateful to our colleague, the neurologist, Peter Doherty was commissioned by Howard Goodman.

Accounts of the ideas and technology that were current immediately prior to the finding of MHC restriction are given in the first edition of Rolf's magnificent book, "Immunology of the Mouse Histocompatibility-2 Complex", and in a review by Katz and Benacerraf[27], which were both published in 1975.

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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 '72, 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. 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, Cole, and Marker and Volkert. They found that the 51Cr-release assay developed by Brunner and Cerottini 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. We decided to use the LCMV 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 51Cr-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 vitro was the essential pathogenetic mechanism. We postulated that acute brain edema, resulting from CTL-mediated damage to the blood–brain barrier, 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 efferent CTLs, but not of T-cell-depleted controls, turned blue.

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. 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 crossed strains available at the JGSMR 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.

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-2b haplotype of the CBA/H. All the LCMV-immune spleen cells from H-2b mice, including F1s, lysed infected L929 cells. By contrast, spleen cells from mice that were H-2d different, namely BALB/c (H-2d) and C57BL/6 (H-2b), 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.

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-2b were indeed able to lyse LCMV-infected, H-2b-compatible target cells. This proved to be more difficult than expected, because the

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**Fig. 1.** Models describing the capacity of F1, H-2b 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 interactions codetermined by the H-2 gene complex (mutuality between either H-2a or H-2b). (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.
other mouse cell lines available in the department (H-2\(^d\) mastocyto
toma P815, or the H-2\(^d\) thymoma EL4) could not be infected with
LCMV and mouse embryo fibroblasts proved to be very 'leaky' for
the \(^5\)Cr label. We thus asked whether cells isolated from the peri-
toneal cavity of mice could be used as a primary source of target
cells, a strategy suggested from Rolf's work with \(L\). \(s\)tetia, which
(as first shown by Mackaness\(^2\)) grows well in macrophages. The
plastic-adherent cells from peritoneal exudates were readily infec-
ted and were labeled with \(^5\)Cr. In October 1973, cross-
section experiments showed that LCMV-immune T cells from H-2\(^d\) mice lysed
LCMV-infected macrophages of H-2\(^d\), 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\(^3\), 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 let-
ter sent back to Canberra summarized data by Gene Shearer show-
ing that trimetaphenil (TNP)-specific CTLs lysed syngeneic TNP-
modified targets better than comparable allogeneic targets; Gene
submitted this to the European Journal of Immunology\(^2\) 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\(^2\) and Herberman\(^2\) and col-
leagues 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\(^3\).
McCullagh\(^2\), and Katz, Hamaoka and Benacerraf\(^1\) had shown sep-
ately 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' sugge-
ted 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 com-
plex, and did not make development of simplifying models an easy
matter\(^2\). Using inbred strains of guinea-pigs in a more direct ex-
perimental system, Shevach and Rosenthal\(^1\) found a tenfold en-
hancement 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 biologi-
cal 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 the intel-
lectually 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\(^2\) (reviewed in Ref. 37). New
and interesting data were constantly emerging from the lab-
oratories of Bob Blanden (cell-mediated immunity), A. Cunningham,
L. Pilarski and P. Bretschler (studying B cells and antibody specific-
ities for heterologous red blood cells and looking for B cells with
sombrero plaques for donkeys and sheep\(^2\)), 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-
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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\(^8\). 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 Pennyy, who juggled work, small children and two obsessed mentors 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.

Rolf Zinkernagel is at the Dept of Pathology, Institut für Experimentelle Immunologie, Universitats Zürich, CH-8091 Zürich, Switzerland; Peter Doherty is at St Jude Children's Research Hospital, 332 North Lauderdale Street, Memphis, TN 38105-2724, USA.

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The preceding article was originally commissioned for Immunology Today by Professor Howard Goodman, whose unique perspective on the history of immunology has been of tremendous value in his role as Editor of our Immunology Yesterday series of reviews.

As with all articles published in Immunology Today, readers are encouraged to submit comments on these important insights into the development of immunological discoveries and ideas.