From influenza to HIV—and back?

Andrew J McMichael

Andrew McMichael recounts the seminal data he and colleagues produced demonstrating that cytotoxic T lymphocytes kill cells expressing antigenic peptides presented on major histocompatibility complex class I molecules.

At the end of 1976 I returned to the UK from Hugh McDevitt's laboratory at Stanford, excited by the phenomenon of major histocompatibility complex (MHC) restriction, newly discovered by Zinkernagel and Doherty[1], and planning to examine its relevance to humans. I was particularly interested in asking whether the restricting role of HLA class I molecules on T cell function might help explain HLA genetic polymorphism. My first idea was to work on herpes simplex virus, but my former PhD supervisor and mentor, Ita Askonas, persuaded me to switch to influenza virus. She had just started to work on T cell immunity in influenza virus infections in mice, with help from John Skehel at the UK National Institute of Medical Research and helped me adapt the mouse protocols to humans. I was soon able to show that recognition of influenza virus–infected cells by cytotoxic T lymphocytes (CTLs) is HLA class I restricted[2]. Then I started to search for responder and nonresponder (to influenza virus) HLA alleles. This search was complicated by the subtle variability in the amino acid sequences of HLA molecules such as HLA-A2, not helped by the fact that my main blood donor (myself) turned out to have a rare A2 subtype, subsequently identified as HLA-A*0205. These small differences in HLA molecules affected T cell recognition of virus-infected cells. Fortunately, Bill Biddison and Steve Shaw at the US National Institutes of Health, also studying influenza-specific CTLs, pioneered identification of the HLA-A2 subtypes, which were then gradually sorted out.

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A puzzling question in those early days was how the influenza virus–specific CTL could be so fastidious about tiny differences in HLA type while at the same time appearing to cross-react broadly with the influenza virus subtypes. This was not solved until Alain Townsend came onto the scene. He first visited me in 1980, sent by Stan Peart, who was Professor of Medicine at St. Mary's Hospital Medical School, to ask for advice on taking temporary leave from his clinical career to work for a PhD. Stan and I introduced him to Ita Askonas, who took him on for the first two years of a PhD. In year three he joined my small group in Oxford. Our original intention was that he would start working on human cells, but this idea was shelved because he had come up with a really exciting finding in Ita's laboratory. She had been one of the first to generate virus-specific CTL clones in mice, nearly all of which appeared to cross-react to all influenza virus subtypes. However, Alain had sought and found a CTL clone, F5, that distinguished between certain influenza A viruses. In collaboration with John Skehel, he used a set of reassorted influenza viruses in which the eight RNA segments of a 1934 and a 1968 virus had been shuffled, to identify the gene segment that encoded the antigen his cloned T cells recognized. Surprisingly, this turned out to the nucleoprotein[3]. A couple of years before, Jack Bennink, in a similar experiment, had identified another internal virus protein, one of the viral polymerases, as a target for CTLs[4]. These were surprising results, because until that time almost everyone believed that CTL must recognize the viral glycoproteins, hemagglutinin and neuraminidase, which are present as intact proteins on the surfaces of infected cells.

Soon after Alain came to Oxford in 1982, we went to see George Brownlee in the Sir William Dunn School of Pathology. George had been the first to sequence cDNA for a full length influenza virus RNA segment[5] and had made cDNA clones for all the virus segments. He generously gave us genes for the nucleoprotein and, for good measure, the hemagglutinin (H3). Alain soon transfected them into mouse L cells, cotransfecting the MHC allele H-2 D^b to match the MHC restricting element for F5. Although the hemagglutinin expressed well, there were problems with the nucleoprotein; it could barely be detected after transfection by immunostaining. After several weeks trying and failing to increase surface expression, Alain performed the critical experiment anyway and found that the F5 clone killed the nucleoprotein-transfected L cells beautifully. The manuscript describing this result was rejected by *Nature* (with one referee's comment that it might only appeal to those with a 'modicum of affection' for T cells), but was readily accepted by *Cell*[6].

Alain Townsend in 1984.

The failure of antibodies to detect nucleoprotein on the surface of infected or transfected cells, which were readily recognized by F5 T cells (and also by polyclonal influenza virus–specific CTLs) provoked much discussion in the lab. Over many beers, we wondered whether the antigens presented in association with class I MHC might be peptides, similar to the peptides presented in association with class II MHC, as shown elegantly by Emil Unanue[7]. To test the idea, Alain worked with Frances Gotch to determine whether fragments of nucleoprotein might be recognized by his clone F5. John Davey in Warwick had broken the influenza nucleoprotein gene into three cDNA fragments, which he transfected into cells to investigate the mechanisms of nuclear targeting. Using the same cDNA, Alain and Frances showed that F5 recognized a nucleoprotein fragment[8].

At that time peptides were hard to make, but John Skehel had made some based on the nucleoprotein sequence. He gave these to Alain and over several more beers we discussed how to give these to target cells, assuming they would have to be taken into the cells before F5 T cells could recognize them. The discussion led not very far, but again Alain just did the experiment that most of us thought would not work. He added the peptides to the wells containing F5 T cells and ^{51}chromium-labeled H-2 D^{b}-positive L cells. That afternoon he looked at the L cells under the inverted microscope and was very excited to see the L cells coming off the plastic bottom of the wells. By early evening the assay had been going long enough to measure chromium release, and sure enough, the target cells had been killed. It is still a puzzle why the experiment (and thousands more like it since) works, because one would think the T cells would also kill one another, but they do not. Thus, the principle that CTLs see short peptide fragments of foreign antigen was established.

Frances and I had some human-specific T cells in culture generated from the blood of Frances's husband, Mike, who made a really strong influenza nucleoprotein–specific HLA-B37–restricted T cell response. We knew the viral antigen specificity because his CTLs lysed HLA-B37–matched target cells infected with recombinant vaccinia expressing influenza nucleoprotein, generously given to us by Bernie Moss and Geoff Smith. The next day, we tested the same peptides in the same way as Alain had done and one worked. Thus the first mouse and human peptide epitopes restricted by class I MHC were found.

We rapidly wrote a paper and sent it off to *Cell*[9]. When it was published, there was early skepticism in certain quarters and reagents had to be sent around so that the experiments could be repeated in other laboratories, but the data were soon confirmed and accepted by all. Then John Skehel introduced us to Don Wiley, who with Pam Bjorkman and Jack Strominger was working on the crystal structure of HLA-A2. They had been puzzled by unidentified electron density that did not fit the HLA-A2 amino acid sequence; our results implied that the density was a mixture of peptides. The paper that followed, describing the structure of HLA-A2 with its peptide binding groove, is one of the most influential in immunology in the twentieth century[10].

Andrew McMichael in 1984.

After these heady days, much followed. Alain started to question how internal viral proteins such as nucleoproteins that are made in the cytosol are degraded to peptides, and how those crossed a membrane to get into the endoplasmic reticulum to join the MHC class I molecules with their empty peptide binding grooves. He proposed that there must be a peptide transporter and that newly synthesized MHC class I might require peptide binding for its stability and export to the cell surface. He, together with Enzo Cerundolo and Tim Elliott in his lab, sought out mutant cell lines that lacked surface expression of class I MHC even though the genes were present, arguing that they might lack the transporter. This led to a collaboration with Klas Karre, who had such a cell line, and Alain and colleagues showed that adding peptide to the cells increased expression of the class I molecules (stabilizing empty class I molecules on the cell surface), making the cells a target for CTLs[11]. Even better, Bob De Mars had previously given us a mutant human B cell line, 721.174, that failed to express HLA class I on its surface, and it behaved in the same way[12]. This cell had a gene deletion in the HLA class II region, prompting a successful search for the peptide transporter in that region, in collaboration with John Trowsdale.

Identification of peptides as antigens for CTLs transformed human T cell immunology. By adding epitope peptides instead of live virus to T cell cultures (together with IL-2) it became possible to grow T cell lines and then clone them very readily. This facilitated precise characterization of virus-specific T cell responses and, subsequently, T cell responses to more complex pathogens and tumors. Around this time, 1987, HIV had emerged as a major health threat. Using recombinant vaccinia viruses expressing HIV gene products and synthetic peptides, Doug Nixon, Frances Gotch and I were able to identify the first epitope in HIV, now usually called KK10, presented by HLA-B27 (ref. 13). Many other epitopes followed, and there are now more than 1,100 mapped and optimized HIV epitopes in the Los Alamos database! Cloning of virus-specific T cells led us into studies of the fine specificity of T cell receptors, including structural studies of HLA-peptide complexes with CD8 and T cell receptors with Yvonne Jones and David Stuart in Oxford.

Having learned how to refold HLA class I molecules with epitope peptides *in vitro*, we were excited when Mark Davis proposed a collaboration with John Bell and ourselves to test a method that he had developed with John Altman of staining human virus-specific T cells with tetramers of MHC molecules. Together, we made HLA-A2–HIV gag tetramers and showed not only that they stained HIV-specific T cells, but that there were a lot more virus-specific cells present than previously suspected[14]. HLA-peptide tetramers are now widely used and have transformed our ability to detect, quantify and characterize antigen-specific T cells. We also used them to identify the NK cell ligand for HLA-E (ref. 15).

In HIV infection, identification of the precise epitopes recognized by CTLs facilitated very detailed examination of these T cell responses and their role in controlling the virus. In 1990, in questions following a talk I gave at a small meeting, Simon Wain-Hobson asked me whether HIV might be able to escape from CTL responses by mutation. Rodney Phillips, Sarah Rowland-Jones, Doug Nixon and I then looked at this question in a small group of patients, sequencing their provirus and asking whether the variations found in the epitopes undermined the T cell responses. This turned out to be the case, and we proposed that HIV was capable of escaping from CTL responses by mutation of epitopes[16]. For a long time this idea was not generally accepted, but in 1997 papers by Philip Goulder in my group[17], by David Price in Rodney Philips' group in Oxford[18] and by Persephone Borrow and George Shaw in the United States[19] showed convincing evi-

Frances Gotch and Andrew McMichael circa 1990.

dence for selection by CTLs of escape mutants. This was followed by a very clear and detailed demonstration of escape from CTLs in acute SIV infection in macaques by David Watkins[20] and then by many excellent papers, in particular from Bruce Walker's group, with sophisticated data analysis from Bette Korber, Simon Mallal and collaborators, that have built up extensive evidence that HIV mutants are selected to evade CTL responses. Now it is recognized that one of the most serious obstacles to the development of an effective HIV vaccine is virus variability and its ability to escape from both humoral and T cell immune responses.

The different types of escape mutation found in HIV, dependent on different HLA types, show one way that HLA type might affect the outcome of a serious virus infection, something that had first interested me in 1977. HLA-B27 and HLA-B57 are associated with better prognosis in untreated HIV infection; data has been accumulating over several years and was recently confirmed and extended in a whole-genome scan for single-nucleotide polymorphisms[21]. The protective effect of HLA-B57 is one of the three strongest disease associations with any class I MHC allele.

Having helped to acquire evidence that CTLs are important in controlling HIV infection, we have worked on vaccines that stimulate HIV-specific CTLs. This approach is likely to be less effective than a vaccine that stimulates broadly neutralizing antibodies, but may be less elusive. This type of translational research is slow, expensive and highly regulated, but the need is great, and we are working with the Center for HIV/AIDS Vaccine Immunology (CHAVI) and the International AIDS Vaccine Initiative as part of a Global Enterprise for HIV Vaccines. Two of the most advanced CTL-stimulating vaccines (from Merck and the NIH Vaccine Research Center) are now in clinical trials to test efficacy.

Our early studies of CTL immunity to influenza sparked off many scientific adventures. By a curious twist of fate, pandemic influenza is now a looming threat. A paper that Frances Gotch and I wrote in 1983 on CTL-mediated protection against experimental influenza virus infection in volunteers[22] has suddenly been noticed again and is being used as an argument for developing 'cross-reactive' T cell vaccines against avian influenza. Using the quantitative assays that we and others have developed for studies of T cell immunity to HIV, we can ask whether preinfection influenza-specific CTL responses protect against natural infection; if so a CTL vaccine aimed at conserved internal influenza viral proteins might be realistic as a protection against pandemic influenza. In a sense I have come full circle.

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