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Legacy of the influenza pandemic 1918: The host T cell response

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ABSTRACT

The influenza virus was instrumental in unravelling critical aspects of the antiviral T lymphocyte mediated immune response. A major finding was the demonstration that CD8 T lymphocytes recognize short viral peptides presented by class I molecules of the major histocompatibility complex. Studies of influenza specific T cells have also led to an understanding of their important role in recovery from influenza virus infection in humans.

The scientific study of Influenza virus, driven by the 1918 pandemic, has led to an understanding of how immunity to this infection evolves and what part the immune response plays in determining the frequency and severity of epidemics and pandemics. At the same time, research on this virus has played a central role in discovery of basic immune mechanisms, particularly in the field of T cell immunity.

CD8 T cells

In the early 1970s R V Blanden introduced the chromium release assay to his laboratory in Canberra to work on cytotoxic T lymphocyte (CTL) responses to intracellular bacterial and virus infections in mice [1]. In 1974 Zinkernagel and Doherty, working alongside Blanden and using this assay, demonstrated major histocompatibility complex (MHC) restriction for the first time [2]. CTL specific for lymphocytic choriomeningitis virus [LCMV] could only lyse virus infected target cells that were compatible for H-2 K or D type of the effector T cells [2]. These results were soon extended to influenza virus in mice by Askonas et al. and Doherty et al. [3–5] and to humans [6]. These studies immediately posed a puzzle. In both mice and humans the CTL showed a very fine specificity for self MHC, not tolerating natural and experimental MHC mutants that differed in just a few, or even single, amino acids [7], but the same T cells cross-reacted with different influenza A virus subtypes. It was universally assumed that CTL must react with the surface glycoproteins, haemagglutinin [HA] and neuraminidase [NA], of influenza A virus, which differ by approximately 30% of their amino acids, between virus subtypes Because of this dichotomy of specificity, some argued that there must be two T cell receptors, one for self MHC and the other for foreign glycoprotein [8].

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The first clue as to the answer came from Jack Bennink and Jon Yewdell who made a CD8 T cell clone that distinguished some influenza viruses from others [9]. Using reassorted influenza A viruses to infect their target cells, they mapped the specificity to the virus polymerase PB2, which is an internal protein on virus infected cells. They suggested that small amounts of PB2 might reach the surface of influenza virus infected cells or that PB2 might influence the expression of another target protein. The prevailing view that T cells could only 'see' intact surface glycoproteins was so entrenched that this demonstration that the antigen was an protein, that is undetectable on the surface of infected cells, attracted little attention at the time. However, Alain Townsend, working in the laboratory of Brigitte Askonas in London, also made a murine CTL clone that could distinguish the 1934 from the 1968 influenza virus and did not recognize the virus glycoproteins [10]. Using reassorted 1934 and 1968 influenza viruses Townsend then showed that the clone must recognize the virus nucleoprotein [NP] [11]. He made a determined but ultimately unsuccessful attempt to demonstrate intact NP on the cell surface and then began to explore the possibility that the CTL clone might see processed fragments of NP in association with MHC class I molecules.

There was a useful but partial precedent because Emil Unanue had just shown that macrophages ingested foreign antigens and processed them to peptide fragments that bound to MHC class II on the cell surface to stimulate CD4 T cells [12,13]. Townsend showed that his CTL clone could recognise histocompatible target cells transfected with one of three fragments of NP [14]. He then tested shorter synthetic peptides (which were very difficult to make at the time) based on the NP sequence. It was not obvious how to get them into, or onto, the cells but he did the simplest possible experiment and simply added the peptides to a mixture of his T cell clone and the ^{51}chromium labelled H-2 compatible target cells and showed that one peptide sensitised the target cells for lysis [15]. Sure enough there was a difference between the 1934 and 1968 virus sequences in this peptide. An hour or so after addition of the T cell clone and peptide to the adherent H-2D^{b} expressing L cells, they curled up and floated off the plastic surface – a Eureka moment! The ^{51}Chromium release results soon confirmed this result [15]. Many in the field were initially sceptical, but the experiment was simple to repeat and the doubters were soon persuaded. Indeed the technology of using short peptides to test antigen specific T cells, and to grow and clone them, underpins nearly all the recent advances in cancer immunotherapy and much else besides.

Very rapidly the finding was extended to humans and other viruses. Strikingly, each MHC type presented different peptides [15,16] including the influenza virus matrix peptide 58–66 presented by HLA-A2 [17]. Around this time [1985–87], Pamela Bjorkman, Jack Strominger and Don Wiley were determining the first crystal structure of HLA-A2, purified from 70 kg of HLA-A2 positive cells in a biochemical tour de force. They had been puzzled by some unresolved electron density at the outer surface of the molecule until the Townsend findings indicated that this must be mixture of short peptides binding into a peptide binding groove [18]. Subsequently a complex of HLA-A2-matrix peptide with the most dominant human T cell receptor was solved [19] [Fig. 1]. This

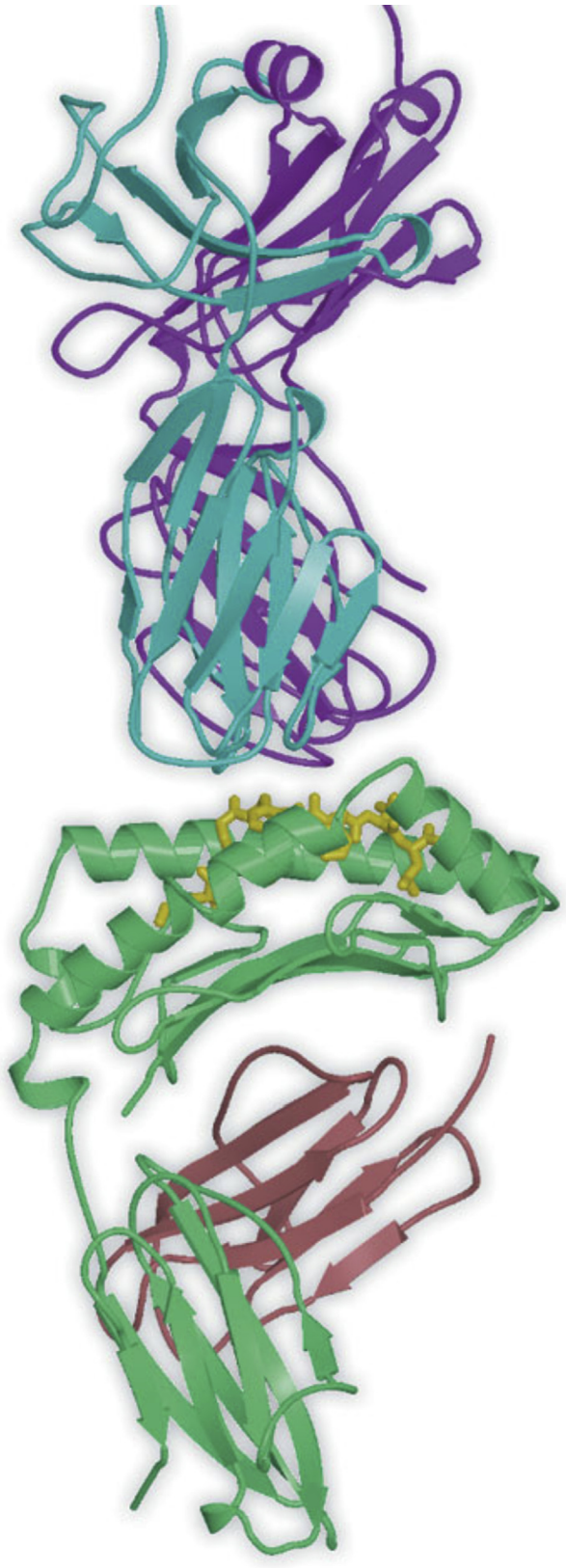

Fig. 1 A resolution of the public T cell receptor BV17AV10.2 complexed with HLA- A*0201 and the influenza virus matrix peptide 58–66 GILGFVFTL. HLA-A*0201 heavy chain green, ß2 microglobulin coral, T cell receptor alpha chain cyan, T cell receptor ß chain magenta. The peptide is shown in yellow. From Stewart–Jones et al., [19].

series of experiments that culminated in the Bjorkman et al., 1987 Nature paper [18] was transformational for immunology; influenza virus was a major player in that discovery.

Alain Townsend, in collaboration with Jonathan Howard, Geoff Butcher, Stephen Powis and John Trowsdale, then went on to work out how the peptide frament, generated in the cytosol of influenza virus infected cells were translocated to the lumen of the endoplasmic reticulum, discovering the transporter associated with antigen processing [TAP] and components of the immune-proteasome [20–22]. Very recent studies have shown how the HLA class I molecule, chaperoned by ERp57, calreticulin and TAP bind Tapasin to form the peptide loading complex. Recently Robert Tampe et al. showed that Tapasin inserts a short 'scoop loop' into the peptide binding groove, holding it open [23,24]. TAP delivers peptides that compete for binding with the scoop loop and those that bind with sufficient affinity to displace Tapasin then enable the MHC molecule to dissociate from the complex and egress the ER. This ensures that the MHC class I molecule binds a limited range of peptides, a possible safeguard against autoimmunity.

Narrow specificities of CTL responses are sometimes matched by rather limited diversity in the T cell receptors of responding T cells. This is particularly true of the HLA-A2 restricted T cell response to influenza matrix peptide, which predominantly uses the βV19 and αV10 TCR chains with very limited polymorphism in the CDR3 regions [25,26]. Thus the CTL response in some 40% of all humans uses a very similar T cell receptor. There must be enormous selection for this TCR which probably has an optimum binding affinity for HLA-A2 and this peptide [19]. At the same time, the similarity of the T cell response likely gives opportunities for virus escape and this has indeed seen across populations [27].

CD4 T cells

Influenza specific CD4 T cells do not have such a central history in immunology although there were notable advances. Jonathan Lamb and David Eckels grew the first human CD4 T cell clones and characterised their HLA restriction [28,29]. It is likely that the different subsets of CD4 T cells (Th1, Th2, Th17, Tregs, TFH) have distinct T cell receptors and are therefore largely non overlapping, so that studies of circulating blood CD4 T cell responses to influenza virus may not correspond to the T cell responses that help B cells in germinal centres of lymph node follicles [30]. TFH cells are only represented within the 5% of blood memory CD4 T cells that express CXCR5 [31], recently confirmed in a study of T cell receptor sharing between blood and tonsil T cell subsets [32]. Thus CD4 T cell responses measured in the blood are more likely to be effector Th1, Th2 and Th17 responses mediating immune inflammatory responses, rather than classical helper T cells involved in maturing antibody responses. They probably do include CD4 T cells that help CD8 T cell responses [33]. In contrast, mouse spleen or lymph node responses are more likely to be representative of true helper TFH responses [34,35]. This makes experimental comparisons between the species difficult. However it is clear that the epitope specificity of anti-HA B cells or pre-existing antibody forming immune complexes [36] influences the specificity of TFH cells, probably by protecting the antibody epitope binding sites from degradation [37].

T helper cells respond to peptide epitopes that are presented by MHC class II molecules. Influenza virus infected respiratory epithelial cells do not normally express these MHC molecules, however adjacent dendritic cells do as antigen presenting cells in draining lymph nodes. Priming of influenza virus specific CD4 T cells is thereby achieved. However, effector CD4 T cells cannot directly attack MHC-II negative cells but there may be indirect cytokine, particularly interferon-γ, and chemokine mediated action stimulated by nearby dendritic cells, or in humans activated T cells. CD4 T cells also recruit innate effectors to the site of infection [38,39]. This should be beneficial but in acute pandemic H1N1pdm influenza virus infection in humans, high levels of influenza virus specific CD4 T cells in the blood was associated with severe disease; possibly harmful recruitment of innate effector cells [40].

The epitopes that CD4 T cells respond to are distinct from those that bind antibody. Indeed helping T cell epitopes can be on a different protein provided the two epitopes are in the same [virus] particle [41]. This means that when a new haemagglutinin variant appears, selected by previous antibody responses in the human population, there may still be cross reactive memory T cells present in many individuals, either seeing more conserved parts of the haemagglutinin or an internal virus protein. This could be one explanation for the phenomenon of 'original antigenic sin' where previous less specific antibodies are boosted when a new virus appears [42]. The suboptimal memory B cells are helped to respond by the well matched and stimulated helper T cells. At the same time the CD4 and CD8 T cell responses may themselves offer a degree of protection against the new virus carrying very similar internal proteins.

Influenza protection by T cell responses

The old dogma has been that antibodies prevent infection and T cells aid recovery. Thus agammagobulinaemic patients are not protected from reinfection with the same strain of influenza virus but recover normally and are not unduly susceptible to severe infection [43,44]. However T cell deficient patients such as those with HIV-1 infection, are not oversusceptible to influenza [45]. This could reflect overlap in protective functions of CD4 [46] and CD8 T cells [47,48].

Early experiments in mice showed that CTL contributed to reduction of virus load and recovery from sublethal influenza infection. The experiments included adoptive transfer of polyclonal and monoclonal CTL, deletion of subpopulations and vaccine induction of CTL in the absence of antibody stimulation [49–51]. Overall, the conclusions were that CTL clear infection, even in the absence of antibody, and that strong CTL responses are beneficial. However there were some experiments showing that circumstances where overactive CD8 T cell responses [52–54] or CD4 Th2 cell responses [55] could be harmful, though often in artificial conditions such as in T cell receptor or haemagglutinin transgenic mice [53]. This could result from lysis of all infected cells in a very widespread infection or from the effects on bystander cells from cytokine release by overactive T cells or from recruited

innate effector cells [40], this would be comparable to a cytokine storm, now a well recognized clinical phenomon, which can be iatrogenic [56] and was likely involved in deaths from acute influenza in the avian H5N1 outbreak [57] and in the 1918 pandemic [58,59]. Although the risk is probably low, such findings imply that efforts to stimulate CTL, CD8 or CD4 T cells, with vaccines should be monitored with caution.

A recently described aerosol infection model in inbred pigs will facilitate further research into lung pathology and virus clearance in a system similar to humans, and indeed a source for reassortment of pandemic viruses [60]. Similar to mice the pigs showed higher levels of virus specific CD8 T cells in the lungs than other tissues during acute infection [61]. This has led to arguments in favour of attenuated influenza virus vaccines delivered intranasally or by aerosol to focus protective T cells at the most vulnerable site [60,62].

The first attempt to show that influenza specific CTL could influence experimental influenza infection in humans was made in the MCR Common Cold Unit in the early 1980s [48] [Table 1]. Volunteers with no protective serum antibody were recruited and challenged with intranasal H1N1 virus, which had returned to infect the children and young adults in 1977. Their subsequent infection, symptoms and virus nasal shedding, 3 and 4 days after infection, was measured and related to pre-existing CTL responses. It was found that those with measurable lytic CTL responses in a short term [7 days] culture assay shed less or no virus. It was not directly determined whether these CTL were CD8 positive but the stronger responses were shown to be HLA-class I restricted. The result implied that the CTL, if present, were eliminating virus infected cells and resolving disease earlier. Although the basis of influenza 'cross-reactivity' was not known at that time, the broader message was that recent prior infection, which stimulated CTL could protect against a new infecting virus, even a new pandemic virus.

The study stood alone for some thirty years until a smaller scale repeat was carried out by Wilkinson et al., in 2010 [46], again on seronegative volunteers challenged with live influenza A virus. A similar result was obtained but CD4 T cell responses were involved [Table 1]. As discussed above, measurements of blood total CD4 T cell responses do not measure classical T helper responses, but are mostly cytokine producing effector T cells, particularly Th1 cells making interferon γ. These T cells are likely part of a broader Th1 response that includes CD4 T cells and CD8 CTL, both acting together to be protective.

During the 2009 pandemic H1N1 influenza outbreak, two groups examined the role of pre-existing T cell immunity to protection, in the absence of antibody protection. Sridar et al. [63] showed in medical staff that pre-existing CD8 T cell responses had significantly less severe illness when infected with the virus [Table 1]. In a large general population cohort, Hayward et al. showed that T cell responses to NP, that were largely mediated by CD8 T cells, were associated with reduced virus shedding in a large community cohort during the pandemic [47] [Table 1]. This pandemic virus was generally relatively benign and many volunteers who were infected showed no overt disease. It was not possible therefore to relate T cell responses to protection against more severe infection. However, in the same pandemic two studies showed that homozygosity for a SNP variant of the viral restriction factor IFITM3 was strongly associated with susceptibility to more severe infection [64,65]. It is not clear how this mutation affects the function of this interferon dependent restriction factor, but the high frequency of this genetic variant in South East Asian populations does not appear to be associated with increase influenza death rates, implying that other protective mechanisms are in play.

Together these studies provide evidence that CD8 T cells and concomitant CD4 Th1 responses can ameliorate mild influenza virus infection in humans and they probably contribute to the large number of subclinical infections seen even in pandemics [47]. Whether these T cell responses could also control severe infection remains uncertain. While there is no reason to think they would not be protective, there could be circumstances of very high virus load where they could be harmful if over-boosted. Without any direct evidence, this type of response has been suggested as the reason why young otherwise healthy adults were particularly prone to death from the 1918 influenza pandemic – the 'W curve' However studies of young previously healthy adults who required intensive care in the 2009 pandemic showed more evidence of overactive innate immune responses in the lungs, rather than T cell responses [66]. Similar observations were made in young people infected with the much more severe avian H5N1 virus in the Far East [67]. However the causes of severe influenza are still not fully understood and there is still good reason therefore to explore the whole immune response longitudinally in patients with severe infections in the future.

Vaccines

The focus of both CD8 and CD4 T cell responses on the more conserved internal proteins of influenza virus has raised the prospect of a universal vaccine that would protect against all subtypes of the virus. Obviously such a vaccine would be invaluable in the 6 month gap between the appearance of a new pandemic virus strain and availability of the first specific vaccine. It has been shown that the standard subunit inert

Table 1 Summary of human studies that show protection by influenza virus specific T cells, either cytotoxic T cells [CTL], CD8+ or CD4+ T cells.

Study reference	Type	Size	Virus	Outcome	Effector	*p* value
[48]	Challenge	63	H1N1	CTL reduce virus shedding	CD8 CTL	0.0069[a]
[46]	Challenge	14 + 9	H3N2 or H1N1	CD4 T cells reduce virus shedding/illness duration	CD4	0.021/0.0008
[63]	Pandemic	342	pdmH1N1	CD8 T cells reduced illness with fever and virus shedding	CD8	0.02/0.06
[47]	Pandemic	1414	pdmH1N1	T cells reduced illness and virus shedding	CD8	0.005

[a] *p* value calculated from chi-square test on whether or not positive [>10% specific lysis] CTL response and detectable nasal virus shedding 3 days after infection.

vaccines do not prime CD8 T cell responses and boost them only weakly if at all [68]. That is not surprising given the requirement for infected cells to prime CD8 T cells and the focus of cross protective immunity on NP, M or other internal proteins that are absent from subunit HA and NA vaccines. However, the cold adapted influenza virus vaccine can boost pre-existing memory CD8 T cells in adults and children and could be useful for stimulating cross reactive T cell immunity [69]. As indicated above there is a possible risk of harm with a CD4 or CD8 T cell inducing vaccine, but that may only be a risk when virus loads are very high and the T cell responses are particularly strong. There have in recent years been many attempts to develop vaccines that stimulate CD8 T cell responses to pathogens, such as the malaria parasite, mycobacterium tuberculosis, HIV and Ebola virus as well as influenza virus [70]. Mostly in these experimental studies, fragments of the pathogen have been used as vectored immunogens, so while the strategies differ the immunogens are similar, though not in sequence. Vaccine vectors include plasmid DNA, encapsulated mRNA, pox viruses and adenoviruses, BCG and Listeria. A number of prime boost strategies have been used with some advantages. For influenza, given that every adult and older children are all primed, a single boost may suffice although heterologous prime-boost could give bigger T cell responses [71].

One issue that needs to be explored further is the site of delivery of the vaccine. As indicated above there is in mice a marked difference in the T cell response at the site of infection compared to distant sites, eg peripheral blood [61]. Thus there could be a case for targeting T cell inducing vaccines at the respiratory tract, particularly the bronchi and lungs for a threatening pandemic virus. The recently describe pig aerosol model and a non-human primate model may be ideal for exploring this [62]. Trying this vaccine route in humans may carry more risk so would likely have to follow extensive safety studies in these animals.

Preparing a vaccine for a future pandemic threat is difficult to fund, given the unpredictability of the emergence and severity of pandemics. For neither the most philanthropic Pharmaceutical company, nor the most far-seeing government, nor charitable agency, is it cost effective to develop a completely new vaccine for it to be shelved for an indefinite period that might be as long as 50 years. Yet if a severe 1918-like pandemic threat emerges in the near future, there would be a deafening demand for a vaccine now. Serious thought needs to go into addressing this dilemma.

Conflicts of interest

None declared.

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