T-Cell Immune Responses and Asymptomatic H5N1 Influenza Infection

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(Approximately 60% of people ill with influenza A virus subtype H5N1 infection die [1], a case fatality rate about 25 times worse than that during the 1918 pandemic [2]. It is hard to even imagine the devastation that would result if such a virus spread widely. However, as discussed by many, the case fatality rate might decline with adaptation to transmission. Even now, H5N1 cases may be underreported (and hence the case fatality rate may be overestimated) because some infections are mild or asymptomatic. Questions about the real frequency of H5N1 infection, and thus the case fatality rate have led to seroprevalence surveys. In areas where H5N1 outbreaks have recently occurred, results show low numbers of seropositive people whose infections must have been asymptomatic or mild [3, 4].

Are such surveys missing H5N1 infections by screening only for antibody? Using antibody as a marker of infection has practical advantages but also limitations, including assay technical issues and low immunogenicity of H5 hemagglutinin (HA) for antibody responses [5]. In this issue of the Journal, the group of Tao Dong at Oxford University (Powell et al) investigated another option. They asked whether assays for T-cell immunity to H5 HA can be used to detect asymptomatic infections. In the past, large-scale population screening for T-cell responses would have been an overwhelming technical challenge. It is still a major effort, but it is now feasible to screen large numbers of human subjects for T-cell responses to overlapping peptide libraries spanning viral antigens.

Powell et al screened a Vietnamese cohort of 747 people with potential asymptomatic H5N1 exposure due to occasional avian and human H5N1 outbreaks nearby in the preceding few years. They used T-cell interferon-γ enzyme-linked immunosorbent spot (ELISPOT) assays with peptide pools based on the HA sequence of A/Vietnam/CL26/2004. T-cell responses to H5 HA peptides were considered positive in 36 donors (relation to H1 or H3 responses undefined), and in 24 of the 36 were at least 2-fold greater than responses of the same donor to H1 or H3 HA peptides. I will call responses meeting this criterion “H5-dominant.” It is not mentioned whether this criterion was defined in advance, or whether this was an exploratory study that evolved as it went along. Among the 3% of donors with H5-dominant responses, a few had very large responses to H5 (highest response >1700 spot-forming units [SFU] per million cells), while a few responses were near background levels (lowest response <25 SFU per million cells).

An additional 111 donors had T-cell responses to H5 that were positive, but lower than to H1 or H3 (called here “H5-nonexclusive”); these were much more common than H5-dominant responses. The H5-nonexclusive responses may also be real and H5-specific, because different T cells may recognize different HAs. Note that the nonexclusive responses to H5 overlap in magnitude with the H5-dominant ones. No evidence was provided that these were instead cross-reactions by T cells seeing public HA epitopes shared by H1 or H3 with H5. Dismissing these responses might be another form of undercounting infections. If we include them, approximately 20% of the cohort responded to H5 peptides.

Healthy controls were drawn from separate populations in the United Kingdom and Vietnam thought not exposed to H5. These controls had no H5-dominant responses, but some may have responded to H5 peptides as well as H1 or H3; information about this possibility was not provided. In a previous study [6], 14 of 20 Vietnamese healthy volunteers responded to H3 peptides and 9 of 20 to H5, with considerable overlap.

Focusing on the small subset of donors with T-cell responses at least 2-fold greater to H5 peptides than to H1 or H3...
(H5-dominant) selects not only for responsiveness to H5 but also for low response to H1 and H3. Low response to H1 and H3 may mean less (or less recent) exposure to H1 and H3 viruses than in other donors. This, in turn, could correlate with lower T-cell responses to other influenza antigens such as nucleoprotein (NP) and matrix, perhaps increasing susceptibility to subsequent infection.

Is it certain that the T-cell responses, or even just the H5-dominant ones, were caused by H5N1 infection? It would be desirable to verify the connection with direct evidence of infection by viral cultures or reverse transcription–polymerase chain reaction. However, the entire cohort would have to be screened for virus shedding on a frequent basis to test for transient asymptomatic infections, and that is not practical. All we can do is examine the evidence provided.

What else other than H5N1 infection could explain the results? Occasionally, T cells induced by one pathogen happen to cross-react with another unrelated pathogen, a phenomenon studied by Welsh, Selin, and colleagues [7] and termed “heterologous immunity.” They have studied cross-reactions of various combinations of pathogens, including human responses to influenza and Epstein-Barr virus [8]. If unidentified prior infections happened to prime T cells cross-reactive with H5, they could be detected by restimulation with H5 peptides and would be expanded in the short-term lines.

The entire cohort was tested for both H5-specific antibodies by hemagglutination inhibition and H5-specific T cells by ELISPOT, and there was little overlap between the individuals positive for antibodies and for T cells. Only 4 individuals had both antibody and T-cell responses, whereas 33 had only antibodies and 20 had only T cells. What could account for this disconnect? For serious, virologically confirmed H5N1 infections, H5-specific antibodies are detectable in most or all patients at suitable times and persist, but they can be missed by testing earlier than 3 weeks after disease onset [9]. Less is known about the kinetics of T-cell responses. H5-specific T-cell responses were seen in only 16% of the group of recovered patients in the Powell et al study, but some samples were collected as long as 4 years after infection. Human T-cell responses to influenza antigens are thought to wane with a half-life of a few years [10]. For H5 HA epitopes, the half-life is unknown, and T-cell screening may not detect infections occurring much earlier. Environmental boosting could have an impact on persistence of responses. Exposure to circulating H1 and H3 viruses would be common, with H5 boosting presumably less common.

The disconnect between antibody and T-cell responses could also be an interesting clue about mechanisms influencing establishment of infection. With human immunodeficiency virus (HIV), some highly exposed individuals remain seronegative but have virus-specific T-cell responses (reviewed in [11]). HIV-specific T cells may mediate early control of virus, reducing its replication, thus minimizing the antibody response. Could this be happening with H5N1? Could abortive infection with H5 virus not well-adapted to humans have stimulated T-cell, but not antibody, responses?

H5 and H2 hemagglutinins are more closely related than H5 with H1 or H3 [12]. Comparing the whole HA sequence to that of A/Vietnam/CL26/2004, a representative H2 sequence in GenBank is 74% identical, compared with 63% for H1 and 39% for H3. For the epitopes identified by the short-term T-cell lines, H2 resembles H5 more closely than does H3 for all 3 epitopes, and more closely than H1 for 2 of them. In the case of the HA5439-56 epitope, H2 is identical to H5. Could the small subset of H5-dominant responders have been exposed to virus subtype H2N2 when it was still circulating prior to 1968? It would be interesting to know the ages of these 24 donors in comparison to the rest of the cohort, the recovered group, and the healthy controls, and to know whether they have antibodies to H2. Any H2N2 infection seems too long ago to account for the responses by itself, but it might have primed for stronger responses to H5 HA.

Now that T-cell assays can be performed in a high-throughput mode, they will be useful in additional ways. With influenza, prior infections with related viruses are common. Many epitopes are not unique to a new strain, but occasional ones are. Gras et al [13] characterized an epitope of NP unique to the 2009 subtype H1N1 pandemic virus, NP418-426. Selective T-cell responses to this variant peptide point to infection with the corresponding virus. This type of response can provide a diagnostic marker, distinguishing infection from vaccination with inactivated vaccine. Also, although T-cell assays currently are too cumbersome and expensive for widespread screening, T-cell testing may prove useful for specialized diagnostic purposes; for example, testing the families of index cases in transmission studies.

The Oxford group carried out an extremely difficult study with the prospect of uncertain returns. Many uncertainties remain, but it is to their credit that they undertook the analysis, and it has raised interesting questions about the use of T-cell assays as diagnostic tests. Several approaches might help resolve the dilemmas discussed above. Perhaps a prospective study in a smaller cohort could include frequent donor testing for virus. Repositories of peripheral blood mononuclear cells retained from vaccine trials could be analyzed to compare induction of T-cell and antibody responses, which would be interesting although not equivalent to wild-type infection. Overall, broader experience with T-cell measurements would be facilitated and standardized if investigators could obtain overlapping peptide libraries from central repositories.

**Notes**

**Acknowledgments.** We thank Graeme Price, Chia-Yun Lo, Carol Weiss, Andrew Byrnes, and
Cecile Viboud for critical review of the manuscript.

Financial support. This work was supported by the Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration (FDA).

Potential conflicts of interest. Author certifies no potential conflicts of interest.

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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