A welcome burst of human antibodies

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A new method allows rapid identification of human monoclonal antibodies from immune or vaccinated individuals.

Several recent technologies for generating human monoclonal antibodies (mAbs) have led to remarkable results in the treatment of cancer and inflammatory diseases[1]. Meanwhile, the use of mAbs to prevent or treat infectious disease has been much more limited[1], with the notable exception of a mAb targeting the respiratory syncytial virus. In a recent study in *Nature*, Wrammert *et al.*[2] describe a rapid route to obtain fully human, high-affinity mAbs to influenza virus. Taking advantage of a huge surge in actively transcribing plasma cells, the authors show how specific antibodies can be cloned from single cells following recent vaccination.

Initially, therapeutic mAbs were generated in mice and used directly in humans. Too often, this practice elicited harmful human anti–mouse antibody responses, which prompted efforts to reengineer rodent mAbs, somewhat laboriously, to look more like human antibodies and thus attenuate these adverse reactions. More recent improvements in mAb isolation and screening technologies have provided access to fully human mAbs.

One major approach for the production of human mAbs is display technology, in which libraries of antibodies are displayed on the surface of a host such as phage, although yeast, bacteria, mammalian cells and ribosomes are also used[1]. The display methods are relatively accessible and also allow for *in vitro* affinity improvement of selected mAbs. A potential drawback of some of these methods is the possibility for unnatural pairings of the antibody heavy and light chain components, although in most cases this is unlikely to be a hindrance.

The advent of transgenic mice engineered to have a repertoire of fully human IgGs[1] gave researchers an alternative route to human mAbs through active immunization, without the protocol restrictions associated with human studies. These transgenic mice cannot exactly reproduce the human antibody response because of repertoire differences and because the genetic background of the murine host affects antigen processing, B-cell ontology and regulation (e.g., tolerance mechanisms). Such differences may be an advantage or disadvantage depending on the research application and on whether breaking tolerance to a particular antigen is desired. At present, these transgenic mice are protected intellectual property and therefore not commonly available.

Another recent methodological advance in the generation of human mAbs involves improved immortalization of human memory B cells in the presence of Epstein-Barr virus, and, importantly, an agent that activates B cells through a toll-like receptor. This approach has been used to identify neutralizing mAbs against a number of pathogens, including SARS coronavirus and H5N1 avian influenza virus[3].

Wrammert *et al.*[2] focused on a distinct population of antibody-secreting cells (ASCs) that give ready access to human mAbs against influenza virus. Several distinct subpopulations of ASCs can be found in the peripheral blood, including those that derive from naive marginal-zone and naive follicular B cells, short-lived plasma cells and plasma cells that are destined for long-term residency in the bone marrow[4]. ASCs can be differentiated from (non-antibody-secreting) memory B cells by probing the levels of various cell-surface markers using flow cytometry.

Wrammert *et al.*[2] sorted for early ASCs by gating cells as $CD19^+$ $CD3^-$ $CD20^{low}$ and then subgating as $CD27^{high}$ $CD38^{high}$. The numbers of their ASCs peaked around day 7 in a short 'burst' after booster immunization and waned rapidly thereafter (**Fig. 1**). Interestingly, the influenza-specific IgG^+ ASC response (reaching 6% of total B cells on day 7) could be clearly distinguished from the influenza-specific IgG^+ memory B cells that peaked later, at day 14 (averaging 1% of all B cells). The high frequency of ASCs in peripheral blood roughly 1 week after booster immunization has been consistently observed with various antigens, such as tetanus toxoid[5]. Indeed the first fully human mAbs from phage were generated from individuals boosted with tetanus toxoid, followed by library construction using peripheral blood lymphocytes isolated 7 days after boosting[6,7].

Wrammert *et al.*[2] also found that the peak ASC response a week after influenza vaccination was 'pauci-clonal'; that is, it consists largely of a few antibody families, each of which is based on a single gene rearrangement and diversified by somatic hypermutation. Thus, an average of 71% of the ASCs sorted by flow cytometry and assayed by ELISPOT were influenza specific. Of further interest is that the flu-specific antibodies had an unusually high rate of somatic mutation: half of the flu-specific ASCs had >20 somatic mutations, whereas only a quarter of the memory IgG^+ B cells were mutated to this degree (the ratio of CDR/FWR mutations was ~2:1).

In a demonstration of their approach, the authors generated a panel of >50 specific anti-influenza mAbs by amplifying the immunoglobulin variable regions from the ASCs by single-cell reverse transcriptase PCR[8], followed by subcloning and finally protein production in 293A cells. In a screen against different influenza strains, most of the mAbs were found to have the highest affinity for the immunizing vaccine strain rather than for strains previously encountered by the host. This finding is at variance with a mechanism called original antigenic sin (OAS)[9], in which the elicited antibodies show higher affinity to previously encountered influenza strains than to the vaccine strain. The results by Wrammert *et al.*[2] rather support a mechanism whereby the ASCs with the highest affinity for antigen are preferentially expanded.

The technique of Wrammert *et al.*[2] is both elegant and convenient for the production of mAbs in a very short time period (<30 days) and should be amenable to many diseases for which human subjects can be followed up the week after a booster immunization. Applications for the human mAbs obtained include therapy for conditions currently treated with polyclonal IgG from serum[2] and for immunocompromised individuals for whom vaccination is not an option.

Wrammert *et al.*[2] identified high-affinity mAbs to several influenza strains. Although not specifically studied, the avian influenza virus H5N1 has repeatedly triggered major international public health concerns arising from reported cases of viral

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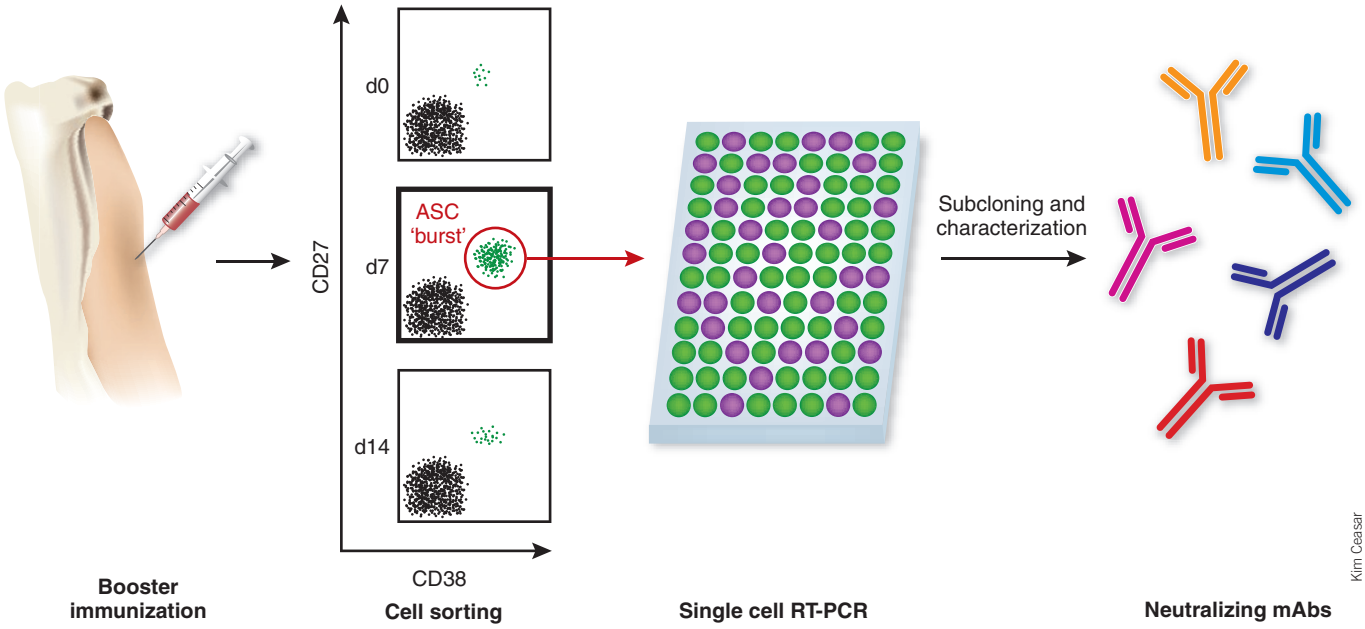

Figure 1 Generation of human neutralizing mAbs from vaccinated individuals. In the study by Wrammert *et al.*[2], human subjects were given a booster immunization against influenza virus, and blood was collected on day 7 post-immunization. Blood cells were specifically sorted by flow cytometry for an early population of antibody-secreting cells (ASCs). Single cells were distributed into multiwell plates and cDNA generated by reverse transcription followed by PCR amplification of the V_H and V_K genes of each cell. Subsequent subcloning and expression of mAbs allowed screening for neutralizing mAbs against a panel of influenza subtypes.

'genetic shift' from birds to humans. Here, human mAbs may also have a niche role in defending against the threat of an outbreak caused by H5N1 influenza or other highly virulent pathogens for which subunit vaccines are not widely available (it is noteworthy that H5N1 is lethal in eggs). However, there are uncertainties about applying the method of Wrammert *et al.*[2] to identify mAbs against chronic and latent pathogens, notably HIV, as well as for cancer and autoimmune diseases, in which the day 7 ASC burst is not readily identifiable owing to chronic B-cell stimulation.

Clearly, it is of interest now to determine how the method of Wrammert *et al.*[2] can be adapted to other pathogens and targets. Applications and improvements of the method will likely come rapidly because of its competitive accessibility and speed and because the demand for human mAbs has never been higher.

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