Milestones... in Investigative Pathology

by William K. Funkhouser, MD, PhD

Edward Jenner and Vaccination: The Road to Elimination of Epidemic Smallpox

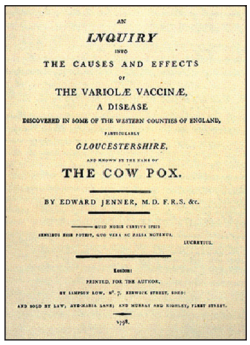
(1) Jenner E. An Inquiry into the Causes and Effects of the Variolae Vaccinae, A disease discovered in some of the western counties of England, particularly Gloucestershire, and known by the name of England, particularly Gloucestershire, and known by the name of the Cow Pox. In. London: Sampson Low; 1798.

When we stand in line waiting for our H1N1 influenza vaccination, and when we have our children vaccinated against previously common childhood infections, we act on the belief that prophylactic immunizations will prevent specific infectious diseases. We make assumptions, e.g. that microbiologists have purified the etiologic agent, that immunologists have identified dominant immunogenic epitopes for B and T lymphocytes, and that clinicians can determine appropriate age, routes, doses, and booster dose intervals to maximize population resistance to the spread of these diseases. We assume that the risk of immunization complications is low compared to the risk of morbidity and mortality associated with the diseases we want to prevent. The net result is that routine prophylactic mass immunization programs have reduced incidence of many of these infections into the realm of rare, call-the-ID-Fellow diseases. This is great news, and gives us a chance to live long enough to procreate and then die of something else. But how did we get to this era in history, wherein epidemics of infectious diseases can be prevented?

Smallpox infection has plagued man for at least 3 millenia. Evidence of probable smallpox infection was found in the mummified Egyptian pharaoh Ramses V from 1157 BC *(www.microbiologybytes.com/virology/Poxviruses.html)*. Recognition of the morbidity and mortality of smallpox in Asia and the MidEast led to empirical prophylactic immunization with smallpox (variola) ulcer debris ('variolation') via respiratory, gastrointestinal (GI), and skin portals by the first millenium AD. The limited appeal of variolation in Western Europe and England was boosted in 1722 when children in the English royal family were variolated. Voltaire wrote in 1733[2] (*www.bartleby.com/34/2/11.html*) that smallpox outbreaks infected an estimated 60% of unvariolated people, and killed a third of those infected. As prophylaxis, variolation reduced smallpox mortality from 20% to around 2%. However, it was neither risk-free nor 100% effective, leading to post-variolation smallpox in some patients and to ineffective prevention of subsequent smallpox in others. Thus, the stage was set for development of a less risky, more effective alternative to variolation.

Dr. Edward Jenner

Dr. Edward Jenner was a rural physician who had been trained to be an experimentalist by Dr. John Hunter. Jenner recalled being variolated as a child, and claimed that it almost killed him. His clinical practice proximity to dairy farmers allowed him to hear firsthand the folklore that farmers and milkmaids exposed to cowpox had a decreased risk of developing smallpox. Just as Dvorak crafted folksongs into the New World Symphony, Jenner crafted folklore into a testable hypothesis about prevention of smallpox through vaccination with cowpox.



AN
INQUIRY
INTO
THE CAUSES AND EFFECTS
OF
THE VARIOLÆ VACCINÆ,
A DISEASE
DISCOVERED IN SOME OF THE WESTERN COUNTIES OF ENGLAND,
PARTICULARLY
GLOUCESTERSHIRE,
AND KNOWN BY THE NAME OF
THE COW POX.
BY EDWARD JENNER, M.D. F.R.S. &c.
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1798.

Cover of Dr. Edward Jenner's 1798 Paper

In his 1798 paper[1] (*www.bartleby.com/38/4/1.html*), he discussed cowpox and smallpox viruses as somehow related, and described the results of a 1796 uncontrolled vaccination trial on children using ulcer debris from cowpox patients. He observed that these children had limited responses to subsequent exposure to smallpox, and interpreted this as a protective effect due to the prior cowpox vaccination. Subsequent larger trials by others obtained confounding results, possibly due to vaccine contamination with smallpox, or to use of skin ulcers due to other organisms. However, preventive vaccination against smallpox was so attractive that mass vaccination programs, e.g. of the Napoleonic army, occurred within the next 10 years -- without a marketing budget.

Dr. Jenner benefited from training that emphasized hypothesis-testing through experimentation. He observed the presence of a clinically similar disease in his clinical practice environment. He noted the current acceptance of variolation in medical practice, and sought to improve the method. The lessons to be learned from Jenner's work are 1) that chance favors a prepared mind (Jenner knew about variolization innoculation techniques); 2) that one should be open to new ideas, however iconoclastic, based on direct observation (Jenner confirmed the folklore about milkmaid's resistance to smallpox); 3) that great benefit is perceived with an effective new approach to a terrible disease (smallpox was so feared that Jenner vaccinated his own son as part of the initial cohort).

During the early 19th century, vaccination against smallpox evolved into direct vaccination, arm-to-arm, from individuals with presumed cowpox infection, without interval purification. The original immunogen was cowpox, but cowpox mimics exist in cattle, and definite cowpox infection was an uncommon infection. Vaccines coming to the United States by the mid-19th century are now known to be vaccinia virus, a related but different species of Orthopoxvirus (*www.ictvonline.org/virus*

Taxonomy.asp?version=2009&bhcp=1). It is unknown whether vaccinia virus evolved from cowpox, pox viruses in horses, or as an attenuated variant of smallpox itself. What is known is that smallpox is a 186 kb double stranded DNA poxvirus that contains 187 major open reading frames[3], and that it generates an active, non-latent infection limited to humans. Forty-nine of the smallpox proteins are conserved in all poxviruses[4]. Vaccinia is closer in size (190kb) to smallpox than is cowpox (224kb). Phylogenetic studies at the protein level suggest that vaccinia and camelpox viruses are the poxviruses most closely related to smallpox [4, 5]. So 19th century smallpox vaccines were emperically selected for effectiveness, and we now know that the most effective live virus vaccines were those most phylogenetically similar to smallpox. (In case you're wondering, chickenpox (varicella-zoster) is a herpes virus, not a poxvirus. It's part of how Infectious Disease keeps the rest of us confused.)

Extension of the concept of disease prevention by vaccination blossomed during the last half of the 19th century, along with cell theory and infectious agent purification and description. In 1880, Dr. Louis Pasteur published his work on the use of 'attenuated' cultures of the diplococcus causing chicken cholera (*Pasteurella multocida*) to prophylactically immunize chickens against lethal infection[6,7]. Pasteur recognized the similarities to Jenner's work 80 years prior, but improved the method by performing controlled experiments on an animal model using a purified immunogen.

To summarize, Jenner had used the clinically similar cowpox ulcer debris to generate cross-reactive immunity to smallpox virus. Subsequent use of vaccinia virus for mass vaccination programs settled on a poxvirus even more similar to smallpox than cowpox. Pasteur introduced use of purified, attenuated bacteria to generate specific immunity to chicken cholera. Each of these approaches capitalized on organisms which could infect, but not kill, the host. Thus, early experiments showed that an ideal vaccine maximizes immunogenicity and minimizes pathogenicity. What neither scientist understood was the mechanism of specific immunity, and how vaccination prophylactically amplified cellular and/or humoral responses to specific organisms.

Experimental studies to understand intra- and inter- species tissue transplantation led to definition of the barcode for individual identity, the gene products of the major histocompatibility complex (H-2, MHC, HLA) locus. Dr. Georg Schone outlined laws of transplantation in 1912, i.e. that xenografts invariably fail, allografts usually fail, and syngeneic grafts usually succeed[8]. Drs. Little and Snell of the Jackson Lab hypothesized that there were multiple histocompatibility loci, each codominantly expressed, and that expression of these MHC gene products by the donor, but not by the recipient, would lead to rejection. They generated congenic mouse strains, differing only by individual major or minor histocompatibility loci, and demonstrated that MHC differences defined the probability of graft rejection.

Dr. Peter Medawar used a rabbit skin transplant model in the early 1940s to demonstrate that the skin graft rejection inflammatory infiltrate is comprised of lymphocytes and macrophages, and that a subsequent second skin graft from the same donor was rejected more rapidly than the first[9]. (The critical role of macrophages as antigen-presenting cells will be a topic for a future Milestones article on Metchnikoff and phagocytes.) Thus, the host immune response to an allograft has a memory for the allo-MHC proteins.

Zinkernagel and Doherty showed in 1974 that a similar T-cell mediated killing process occurs during recognition of viral infected cells[10]. Using lymphocytic choriomeningitis (LCM) virus as a model infectious agent in mice, they demonstrated that lymphocyte killing was **restricted** by recognition of their own MHC molecules on macrophages and other virally infected cells. T lymphocytes therefore activated only to (self MHC + viral antigen). Medawar's T lymphocyte response to allo-MHC in rabbit skin grafts could then be viewed as a version of (self MHC + antigen). Subsequent work showed that the antigenic proteins are not exhibited whole, but rather must be cut up (processed) and bound as short polypeptides to self MHC class I and class II molecules (presented) on the cell surface (reviewed in[11,12]). The model is that MHC class II$^+$ cells (antigen presenting cells) first activate viral epitope-specific 'helper' (CD4$^+$) T lymphocytes, which secrete lymphokines that in turn allow activation and proliferation of viral epitope-specific 'cytotoxic' (CD8$^+$) T lymphocytes and viral epitope-specific B lymphocytes. Infected MHC class I$^+$ cells are then recognized by these viral epitope-specific cytotoxic T lymphocytes, and free virus can be bound and cleared by viral epitope-specific immunoglobulin. Thus, transplantation studies led to an understanding of the barriers posed by MHC, and that allo- and xeno- transplantation barriers are perceived by the specific immune response in the same way as xeno-infectious agents, i.e. as non-self.

A subset of the T lymphocytes activated in response to the viral antigens is long-lived, and retains its specificity for the cognate antigen. For the anti-vaccinia response, circulating CD4+ T lymphocytes can be activated correctly over 35 years after childhood immunization against smallpox, with one limiting dilution estimate of 3 CD4$^+$ T cells per 10^6 CD4$^+$ lymphocytes [13]. Like the allograft response, the host response to virus has a memory, and it is these memory cells that allow the individual to mount a more rapid secondary response to the infectious agent.

In theory, a robust vaccine would be processed and presented by all MHC class I and II haplotypes, would trigger memory T and B lymphocyte formation, and would not require booster immunization. In practice, insufficient processing/presentation, insufficient T cell numbers, or insufficient T cell function (HIV, iatrogenic immunosuppression) could each lead to an inability to respond to antigen and generate memory of the antigen. This could cripple the host's ability to respond to the infectious agent, including responding to attentuated live organism vaccines. In fact, some children vaccinated with vaccinia virus developed progress vaccinia, occasionally with bad complications[14]. This would be expected, based on the intricate requirement for protein processing and polypeptide presentation on MHC molecules to T lymphocytes. Fortunately, most individuals can process and present whole-organism vaccine proteins, and can amplify a virus-specific immune response and memory cell formation before the actual pathogen is contacted.

If live organism vaccines pose a real but unpredictable risk of clinical infection and death, then perhaps vaccine engineers can identify polypeptides that are produced in the organism, and which would act as dominant epitopes for T and B lymphocyte activation. Individuals able to present these polypeptide epitopes sufficient to trigger T cell activation should be protected from subsequent infection by earlier, more intense secondary response via memory lymphocytes and circulating

antibody. The challenge will be to identify sets of dominant epitopes which can be presented by all HLA haplotypes in the outbred human population.

Whether whole-live, whole-killed, or as dominant polypeptides, the timing of the secondary response is critical. For smallpox, new exposure starts the clock ticking, with the 7-10 days required to mount a primary specific immune response too close for comfort to the 12 day incubation period before the first viremia. When this race occurs between a non-immune individual and the smallpox virus, 30% of infected individuals die. The more immediate secondary response, and the presence of circulating anti-viral antibody, allow more of the vaccinated population to survive. Therefore, prophylactic immunization prepares the individual's specific immune system to respond promptly and decisively to subsequent infection.

Can mass vaccination reduce disease incidence to zero? The World Health Organization (WHO) decided to eradicate smallpox in 1958. WHO-sponsored vaccinia vaccination programs against smallpox in the 1960s eradicated clinical smallpox from the earth, with the last reported case of non-lab-acquired smallpox in October 1977. As of 1980, smallpox existed only in research labs in the US and the USSR. (We can only hope that these stocks are secure, because none of our children have been vaccinated against smallpox.) And nature continues to test our ability to keep up with viral mutation rates and manufacture specific vaccines, as evidenced by the annual challenge of predicting the Hexoseaminidase-Neuroaminidase combination for influenza vaccines. New viruses also adapt to humans, e.g. HIV, which began to be clinically significant just as clinical smallpox was extinguished.

Dr. Jenner was, and we are, fortunate that smallpox virus had no non-human reservoir, had no asymptomatic human carrier state, and that it had a close poxvirus relative (cowpox) with a local occupational association, similar skin signs of infection, and viral epitopes which led to a cross-reactive specific immune response to smallpox. Serendipity, indeed! Pasteur improved the method through use of attenuated variants of the purified agent itself. Jackson Labs' inbred mouse strains led us to understand class I and class II MHC expression, i.e. the molecular basis of self. Medawar showed that the immune response to allograft has memory which speeds the secondary exposure to antigen. Doherty and Zinkernagel demonstrated the MHC-restricted nature of T cell activation to macrophages and other virally infected cells, and Demkowicz documented $CD4^+$ T lymphocytes memory lasting decades after childhood vaccination with vaccinia. Convincing bench, clinical, and epidemiologic data, along with international commitment, allowed elimination of epidemic smallpox on our planet in our lifetime. We did it, and we thank you, Dr. Jenner.

P.S.: If you're wondering what the Latin quote on the cover page of Dr. Jenner's 1798 paper ("Quid nobis certius ipsis sensibus esse potest, quo vera ac falsa notemus") means, it translates as "What, more certain than our senses themselves, can there be, by which we indicate truths and falsehoods?"

Web-based resources for additional reading:

www.nlm.nih.gov/exhibition/smallpox/
www.microbiologybytes.com/virology/Poxviruses.html
www.bt.cdc.gov/agent/smallpox/vaccination/live-virus.asp

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