Year of the ox

Yann Echelard

High levels of human polyclonal antibodies have been produced in a transgenic large animal.

With a market now worth well over $2 billion in the United States[1], human polyclonal antibodies purified from thousands of plasma pools have become standard therapy for many viral infections and immune disorders[2] and for neutralization of toxins[3]. Despite their clinical potential, however, the use of polyclonal antibodies remains limited by issues related to their supply, cost and safety[4]. In this issue, Kuroiwa *et al.*[5] bring us a step closer to large-scale production of relatively homogenous recombinant polyclonal antibodies, which could alleviate these problems and expand application of this therapeutic modality to new indications[6].

Unlike monoclonal antibodies, which recognize a single epitope, polyclonal antibody preparations bind multiple epitopes on the disease-causing agent and can thereby neutralize distinct variants of toxins or infectious particles, making them the agents of choice for treating certain medical emergencies and acute illnesses. Hyperimmune globulins—sourced from human or animal donors with high titers of antibodies against specific antigens—are in high demand to curb immunosuppression associated with transplants; prevent Rh hemolytic disease; treat and prevent infections such as hepatitis B, hepatitis A, rabies, respiratory syncytial virus, cytomegalovirus and varicella-zoster; and neutralize toxins, including diphtheria, botulism, digoxin and snake and spider toxins[2,3].

The ability to produce human antibodies in mice expressing human immunoglobulin genes has long been appreciated[7], and mice now provide a convenient source of hybridomas for generating candidate therapeutic human monoclonal antibodies. However, the small body size of mice makes them unsuitable for synthesizing large amounts of hyperimmune globulins. Aiming to translate this approach to a large animal, Kuroiwa and colleagues previously expressed the human immunoglobulin heavy chain and λ-light chain from a human artificial chromosome in cloned cows[8]. Although these animals did produce human antibodies, the levels were too low to be of practical utility as the active endogenous immunoglobulin loci suppressed expression of the human genes[6,8].

The feasibility of introducing human immunoglobulin genes and knocking out endogenous immunoglobulin genes in cattle has been far from certain. First, it is not straightforward to perform multiple genetic modifications in large animals: embryonic stem cell lines are not available and generation intervals are long (around three years in cattle). Successive rounds of transfection and selection in primary cells, which have a limited life span, each followed by somatic cell nuclear transfer (to regenerate the cell line) are necessary to introduce the targeting constructs and the human immunoglobulin loci. Second, the accumulation of epigenetic errors caused by successive rounds of nuclear transfer has been reported to compromise the viability of offspring. Finally,

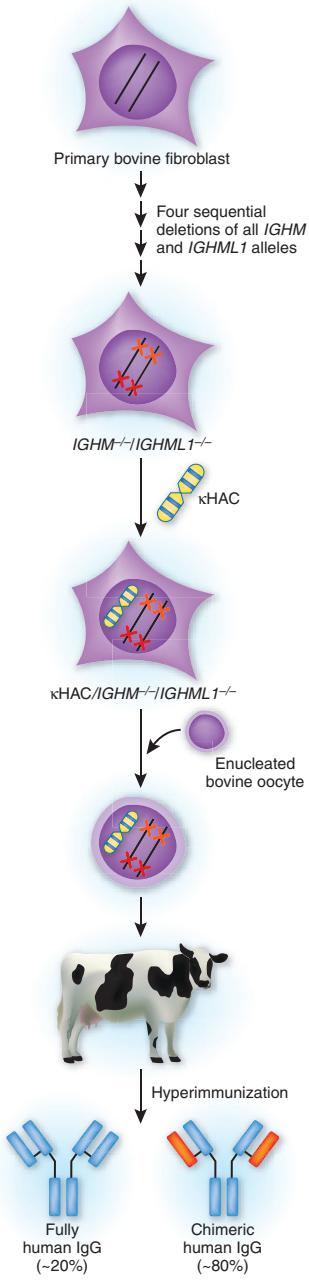

Figure 1 Cattle capable of producing human polyclonal antibodies are produced by multiple cycles of transfection, selection and nuclear transfer[5]. Each of the four bovine IgM heavy chain alleles is knocked out by homologous recombination followed by somatic cell nuclear transfer to extend the life span of the selected cell lines. An artificial chromosome carrying the human immunoglobulin heavy and κ-light chain loci (κHAC) is transferred to the multitargeted bovine cell lines by microcell-mediated chromosome transfer, and three additional nuclear transfer steps are performed to obtain a healthy transgenic calf with the κHAC/*IGHM*$^{-/-}$/*IGHML1*$^{-/-}$ genotype. Vaccination of this animal with an antigen of interest produces ~20% fully human antibodies and ~80% chimeric antibodies (bearing human heavy chains and bovine light chains).

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it has been unclear whether human immunoglobulins alone could support bovine humoral immunity in the absence of endogenous bovine immunoglobulins.

These formidable challenges make the achievement of a transgenic calf expressing high levels of human antibodies all the more remarkable. Kuroiwa *et al.*[5] focused first on successively inactivating all the bovine IgM heavy chain genes (**Fig. 1**), whose expression is essential for B-cell development. Because ruminants, unlike mice and humans, have two functional IgM loci (*IGHM* and *IGHML1*), four alleles had to be targeted to knock out IgM heavy-chain production. In the next step, the $IGHM^{-/-}$ $IGHML1^{-/-}$ fibroblasts were transfected with an artificial human chromosome (κHAC) bearing the unrearranged human heavy and κ-light-chain loci (**Fig. 1**). In the end, after seven rounds of cloning, a healthy transgenic calf, with all bovine IgM heavy chain alleles inactivated and bearing the human artificial chromosome, was obtained. This animal expressed 60-fold more human immunoglobulins than animals described previously[8]—a yield that is potentially competitive from a cost perspective with producing nonhuman hyperimmune globulins.

Vaccination of the calf with anthrax-protective antigen yielded high titers of anthrax-specific immunoglobulins. Although ~80% of serum IgGs were functional chimeric antibodies comprising human heavy chains and bovine light chains, the remainder were fully human (**Fig. 1**). Once purified, the hyperimmune globulins fully protected mice challenged with anthrax spores and in an *in vitro* toxin neutralization assay outperformed a control anthrax hyperimmune globulin preparation derived from human donors.

Importantly, the calf's immunization response was similar to that of wild-type cattle, confirming that the human immunoglobulin loci can support the humoral response in the absence of bovine IgM. Other transgenic calves produced in this study appeared to produce similar levels of human IgGs, suggesting that a herd of cattle with this genotype could provide an abundant source of human hyperimmune globulins. Nevertheless, extensive purification will be necessary to obtain preparations containing only fully human IgGs, which will increase production costs. Knocking out the bovine Igλ locus, which contributes ~90% of light chains in cattle, in this line could further increase the proportion of fully human immunoglobulins and improve process yields.

Although the seven years that have elapsed since this group reported transchromosomic calves expressing human immunoglobulin loci[8] might seem like a long development time, it is worth remembering that the line generated in the present study[5] or subsequent lines could support production of multiple products. Each new hyperimmune globulin product would be dependent on the antigen used in the immunization protocol, rather than the bovine line or the purification process. Scale-up should be relatively straightforward, although this might require cloning rather than natural breeding.

It is still too early to confidently predict the commercial success of human hyperimmune globulins from transgenic cattle. Uncertainties remain concerning, for example, the impact of purification on production costs and the feasibility of using somatic cell nuclear transfer to generate large numbers of animals. Clinical studies—the costliest and riskiest aspect of drug development—must also be completed. But given the flexibility and scalability of using transgenic large animals, this approach may be well placed to compete with traditional human- and animal-derived intravenous immunoglobulins, hyperimmune globulins, and monoclonal and polyclonal antibodies produced in cell culture[9] in applications spanning infectious diseases, oncology, neurological conditions and immune modulation. As we enter the Chinese year of the ox, it seems fitting to look forward to clinical trials of polyclonal antibodies obtained from transgenic cattle.

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One photon up, one photon down

Enrico Gratton & Michelle Digman

A microscopy technique based on stimulated Raman scattering achieves label-free imaging with very high sensitivity.

Identifying different molecular species in microscopic images is still a considerable challenge in many areas of biology. Most commonly, especially in experiments on live cells and tissues, what is detected is not the native molecule but a fluorescently labeled analog, which is assumed to mimic the behavior of the unlabeled molecule. In a recent *Science* paper, Xie and colleagues[1], describe a new techniqúe, stimulated Raman scattering (SRS) microscopy, that is capable of imaging unlabeled molecules in live cells and tissues with diffraction-limited resolution and high sensitivity.

Since the 1920s, when Chandrasekhara Raman[2] first explained the loss of energy to vibrations from a beam of monochromatic light traversing a liquid sample, investigation of the quasi-elastic interactions of light with matter has become a major technique for analyzing the vibrational spectrum of molecules in the condensed state and the composition of biological samples[3]. The advent of the laser in the 1960s and the introduction of resonance Raman scattering, which increase the sensitivity to specific vibrations near a chromophore, made this a relatively simple and accessible method. The appeal of this approach in microscopy is that the molecular vibrations excited by the Raman effect are exquisitely dependent on local molecular arrangements and therefore serve as a fingerprint of individual molecules or classes of molecules.

Until now, the small intensity of Raman scattering has meant that only techniques based on coherent anti-Stokes Raman scattering (CARS) have had the sensitivity necessary for diffraction-limited microscopy[4]. In the CARS effect, two laser beams impinge on the sample. The pump laser excites a vibration and the probe laser produces the anti-Stokes transition (that is, addition to the probe laser of energy contained in excited molecular vibrations), resulting in a new emission wavelength different from those of the pump and probe lasers. This new wavelength characterizes the energy of the

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