thereby initiate the sequence of events in the uterus that results in delivery of the infant.

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7. The urine samples were obtained at first voiding from full-term (38 to 42 weeks of gestation) newborn infants immediately after delivery. Such urine had been formed while the infant was in utero and was therefore fetal urine. All infants were healthy and of appropriate size for gestation. Specimens were obtained during spontaneous urination or by gentle suprapubic pressure. The samples were stored at −20°C until tested.
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Bovine × Mouse Hybridomas That Secrete Bovine Immunoglobulin G_1

Abstract. *The interspecific fusion of normal bovine lymphocytes with a nonsecreting mouse hybridoma produced stable cell lines secreting bovine immunoglobulins. One of these lines has continued to secrete immunoglobulin G_1 (5 to 10 micrograms per milliliter) for over 16 months. The bovine × mouse hybrid cells can be expected to provide bovine monoclonal immunoglobulins for sequencing studies and for use as serological standards as well as to provide messenger RNA for cloning bovine immunoglobulin genes.*

The availability of monoclonal immunoglobulins (Ig's) first from myelomas (*1*, *2*) and recently from hybridomas (*3*, *4*) has been of critical importance to the development of our current picture of the structure of human and mouse Ig's and their genes. Antibody-secreting hybridomas have been produced by intraspecific fusion of myeloma cells with normal cells of the B lymphocyte lineage (*5*–*8*) as well as by interspecific fusion of mouse myeloma cells with B lineage cells of nonmurine species (*9*, *10*). However, cell lines secreting monoclonal bovine Ig, derived from normal cells of the B lymphocyte lineage, have not been demonstrated. Hence the field of veterinary immunology has been without a source of bovine monoclonal Ig's for sequence studies and for the preparation and testing of class-specific antisera.

Table 1. Specific immunoabsorption of the hybridoma product. The hybrid cells (1×10^7) were cultured in 2 ml of methionine-free DME medium containing 1 percent fetal bovine serum and 0.5 mCi of [^{35}S]methionine for 6 hours. The culture supernatant was used in a binding assay. Six milligrams of the affinity-purified mouse monoclonal antibody DAS-1, specific for bovine IgG and IgM, was coupled to 1 g of CNBr-activated Sepharose 4B and suspended in 15 ml of PBS containing 0.05 percent polyoxyethylene sorbitan monolaurate (Tween 20). To 1 ml of this suspension was added 100 μl of the labeled culture supernatant, and the mixture was agitated slowly for 30 minutes. The ability of whole bovine serum or whole mouse serum to block the binding of the labeled product in the culture supernatant to DAS-1 was tested by adding 50 μl of each of these sera, respectively, to the DAS-1–conjugated Sepharose prior to the addition of the culture supernatant. The reacted Sepharose was then washed three times by the addition of 10 ml of PBS containing 0.05 percent Tween 20 and centrifuging at 400*g* for 5 minutes. The Sepharose pellet was then counted in a scintillation counter.

Sample treatment	Radioactivity (count/min)*
No incubation	41,610 ± 60
Incubation with bovine serum	3,473 ± 60
Incubation with mouse serum	40,226 ± 370

*Mean ± standard deviation.

We report here the interspecific fusion of a mouse hybridoma with normal bovine spleen cells, resulting in stable hybrid cell lines that secrete monoclonal bovine Ig molecules. Interspecific hybridomas were produced because HAT (hypoxanthine, aminopterin, thymidine)-sensitive bovine myeloma cell lines are not available (*11*). These hybridoma cell lines manufacturing monoclonal bovine Ig will be useful (i) for the production of monoclonal bovine Ig for sequencing studies and as reference reagents for the serology of bovine Ig; (ii) for the production of continuous cultures of hybridomas secreting bovine Ig of predefined antigenic specificity by use of bovine spleen cells from appropriately immunized donors as fusion partners; and (iii) as a source of messenger RNA (mRNA) for the cloning of bovine Ig genes.

Bovine × mouse hybridomas were prepared by polyethylene glycol (PEG)–assisted fusion of normal bovine lymphocytes to the mouse cell line SP-2/0 (*12*), a mouse × mouse hybridoma which does not secrete or internally produce mouse Ig. Bovine lymphocytes were obtained from the spleen of an adult female Holstein cow by first mincing six randomly selected cubes of spleen (~ 2 cm) with scissors and grinding the mince between the frosted faces of microscope slides in serum-free growth medium [50 percent RPMI 1640 and 50 percent Dulbecco's modified Eagle's medium (DME)]. The lymphocytes were washed twice in serum-free growth medium, mixed in a 1:1 ratio with 1×10^8 SP-2/0 cells, and centrifuged at 400*g* for 15 minutes at room temperature. The pellet was then slurried in 2 ml of 52 percent polyethylene glycol 1540 in serum-free growth medium and incubated for 2 minutes at 37°C. The mixture was then diluted over the course of 10 minutes to 50 ml by the addition of serum-free growth medium and centrifuged for 15 minutes at 400*g* at room temperature. The fused cells were resuspended in HAT (*13*) medium (45 percent RPMI 1640, 45 percent DME, 10 percent γ-globulin–free horse serum, 2 m*M* glutamine, penicillin (10 U/ml), streptomycin (100 μg/ml), 1×10^{-4}*M*

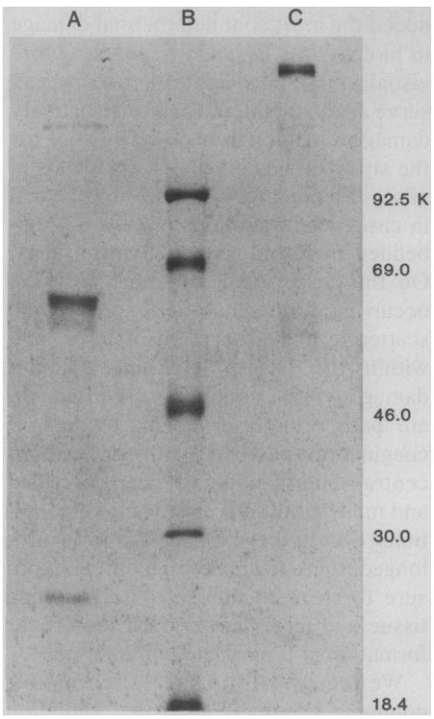

Fig. 1. Autoradiograph of labeled product secreted by the hybrid cell lines analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (*17*). Hybrid cells (1×10^7) were cultured in 2 ml of methionine-free DME medium containing 1 percent fetal bovine serum and 0.5 mCi of [^{35}S]methionine for 6 hours. The supernatant was applied on 7 percent polyacrylamide gel slabs before reduction (lane C) and after reduction (lane A). Lane B shows $^{14}CH_3$-labeled molecular weight standards.

hypoxanthine, $4 \times 10^{-7}M$ aminopterin, and $3 \times 10^{-5}M$ thymidine) and distributed in 0.2-ml portions to the wells of microtiter dishes. We obtained 63 single hybrid clones between 14 and 21 days after fusion.

Supernatants of the hybrid clones were assayed for production of bovine Ig by a competition radioimmunoassay (*14*) with the use of affinity-purified polyclonal rabbit antibody to bovine λ light chain. For initial screening, polyvinyl chloride microtiter plates were coated with the antibody to λ light chain. The antibody [20 μl; 50 μg/ml, in phosphate-buffered saline (PBS)] was added to each of the wells, and the dishes were placed in an incubator at room temperature for 1 hour and then washed three times with PBS containing 1 percent bovine serum albumin (PBS-BSA). Supernatants were tested for the presence of bovine Ig by adding 20 μl of the supernatant and 20 μl of ^{125}I-labeled bovine IgG (specific activity, 10 μCi/μg) to the antibody-coated wells. Wells that received 20 μl of growth medium, which had no contact with the hybrid cells, and 20 μl of ^{125}I-labeled bovine IgG served as controls. After 1 hour incubation, the wells were washed three times with PBS-BSA and counted in a γ counter. The count is inversely proportional to the bovine Ig content of the supernatent. Initial screening of the 63 single hybrid clones revealed 21 clones secreting bovine Ig. After three cycles of subcloning, three cell lines continued to secrete bovine Ig and one, LHRB-1, was selected for intensive investigation. This cell line was shown to secrete whole bovine IgG_1 molecules, and this secretion continued for 16 months at a rate of 5 to 10 μg of IgG_1 per milliliter per 5×10^5 cells per 24 hours.

A panel of monoclonal antibodies with known specificities for bovine Ig isotypes was used to determine that LHRB-1 was secreting bovine Ig heavy chains and to establish the isotype of the heavy chain. The monoclonal antibody DAS-1 (*15*), which binds to bovine IgG_1, IgG_2, and IgM but fails to react with IgA, was used to establish that the product of LHRB-1 is a bovine Ig. As reported in Table 1, the [^{35}S]methionine-labeled product of this cell line binds to DAS-1–conjugated Sepharose. This binding was inhibited by whole bovine serum but not by whole mouse serum. In a similar experiment, the labeled product did not bind above background levels to affinity-purified sheep antibody to mouse Ig coupled to Sepharose. These results indicate that the hybridoma product is a bovine Ig. Two other monoclonal antibodies, DAS-2 (*16*), which is specific for bovine IgG_2, and DAS-6 (*15*), which is specific for bovine IgM, were used in concert with the aforementioned DAS-1 in competition radioimmunoassays to establish the isotype of the Ig secreted by LHRB-1 as IgG_1 (Table 2). The hybridoma product in the culture supernatant inhibited the binding of ^{125}I-labeled IgG_1 but not ^{125}I-labeled IgG_2 or ^{125}I-labeled IgM to their respective antibodies, indicating that the product was IgG_1.

The gel in Fig. 1 demonstrates that the product secreted by the bovine × mouse hybridoma is an Ig molecule in which light chains are covalently bound to heavy chains by disulfide bridges. Although the reduced product of LHRB-1 yields heavy and light chains with apparent molecular weights of 62,000 and 26,000 daltons, respectively, the unreduced molecule does not yield heavy and light chains.

Continuous cultures of bovine × mouse hybrid cell lines, even though chromosomally less stable than intraspecific hybridoma cell lines (that is, mouse × mouse hybridomas), supply homogeneous bovine Ig in virtually unlimited quantities. Such Ig's can be used as serological standards and as antigens of choice for the production of polyclonal and monoclonal antisera to bovine Ig isotypes for use in diagnostic assays. Monoclonal bovine × mouse hybrid cell lines may provide material that will open new areas of investigation of the bovine immune system that were heretofore inaccessible.

Table 2. Determination of the Ig class and subclass. Culture supernatant from hybrid cells was used in a standard competition radioimmunoassay (*14*) to determine the Ig class and subclass of the product of the hybrid clone. Affinity-purified mouse monoclonal antibody to bovine Ig (DAS-1, DAS-2, and DAS-6) was immobilized on polyvinyl chloride microtiter wells, by coating with 20 μl of a solution of the antibody in PBS (50 μg/ml). The monoclonal antibody DAS-1 bound to bovine IgG_1, IgG_2, and IgM, whereas DAS-2 and DAS-6 were specific for bovine IgG_2 and IgM, respectively. Duplicate samples of 20 μl of either the culture supernatant or the growth medium, which had no contact with the cells, were added to the microtiter wells. Simultaneously, 10 μl of ^{125}I-labeled bovine IgG_1, IgG_2, and IgM (specific activity, 10 μCi/μg) were added to the microtiter wells coated with DAS-1, DAS-2, and DAS-6, respectively. After 1 hour of incubation and three washing cycles with PBS-BSA, the wells were cut and the radioactivity counted. The percentage inhibition of the binding of the ^{125}I-labeled antigens by the hybridoma product in the culture supernatant was calculated as the radioactivity given by wells that received culture supernatant subtracted from the radioactivity given by wells that received growth medium divided by the radioactivity given by wells which received growth medium × 100.

Immobilized monoclonal antibody	Antigen specificity	^{125}I-labeled antigen	Inhibition (%)
DAS-1	αIgG_1, αIgG_2, αIgM	IgG_1	60
DAS-2	αIgG_2	IgG_2	0
DAS-6	αIgM	IgM	0

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Selective Photothermolysis: Precise Microsurgery by Selective Absorption of Pulsed Radiation

Abstract. *Suitably brief pulses of selectively absorbed optical radiation can cause selective damage to pigmented structures, cells, and organelles in vivo. Precise aiming is unnecessary in this unique form of radiation injury because inherent optical and thermal properties provide target selectivity. A simple, predictive model is presented. Selective damage to cutaneous microvessels and to melanosomes within melanocytes is shown after 577-nanometer (3×10^{-7} second) and 351-nanometer (2×10^{-8} second) pulses, respectively. Hemodynamic, histological, and ultrastructural responses are discussed.*

Many biomedical applications of lasers have been developed (*1, 2*). The first effect of light on tissue is the absorption of photons, which leads either to photochemical reactions or to significant heating. With few exceptions (*3*), biomedical lasers use a variety of thermal effects (*4*). Rapid localized heating causes large thermal transients and shock waves which may propagate, causing mechanical damage. Many enzymes are heat-labile. Above 60° to 70°C, structural proteins including collagens are also denatured (*5*). Above 70° to 80°C, nucleic acids are denatured and membranes become permeable. Thus, essentially any mammalian tissue heated to 70° to 100°C may suffer protein denaturation, leading to "coagulation necrosis." Coagulation necrosis is useful for causing hemostasis due to the denaturation of plasma proteins and the closing of vessels. Above 100°C, vaporization of tissue water with rapid volume expansion followed by carbonization of the dry mass occurs. Rapid vaporization is useful for physically separating or ablating tissues.

Although the mode of damage is important, it is the spatial confinement of heating which mainly dictates which cells or tissues will be affected. Laser "microbeam" microsurgery has produced the most confined thermal damage in biology (*6*). Essentially any structure visualizable under light microscopy can serve as the target and can be selectively damaged. Although a powerful tool for the study of single cells or organelles in vitro, laser microbeams are impractical in cases where millions of cells are embedded in turbid, intact, living tissues. On this larger scale, thermal diffusion occurring during and after exposure and scattering and absorption of laser light within the tissue determine whether damage will be confined to the immediate path of a laser beam. Craters of coagulation necrosis with or without central vaporization have been described and modeled for a great variety of conditions (*7*). If the exposure time is prolonged, there is ample time during exposure for heat to diffuse to surrounding tissue and larger craters are seen. Scar formation is typical of such injuries.

We present here a simple scheme for confining thermally mediated radiation damage to chosen pigmented targets at the ultrastructural, cellular, or tissue structural levels. Experimental verification is shown for two biologically interesting targets—blood vessels and melanocytes. The confinement of damage can be as precise as with microbeam techniques, but millions of targeted structures are damaged simultaneously in vivo without precise aiming. This may be particularly useful in turbid tissues, which unlike the eye, limit the precision with which isolated structures can be exposed. Tissues between targeted structures, including overlying or immediately neighboring cells, are spared, potentially reducing widespread destruction and nonspecific fibrosis. There appear to be few fundamental limitations to applying the approach in various tissues and to a wide range of targets. We call the technique selective photothermolysis (SP).

This technique relies on selective absorption of a brief radiation pulse to generate and confine heat at certain pigmented targets. An absolute requirement is that the targets have greater optical absorption at some wavelength than their surrounding tissues. This requirement can be met either by choosing endogenously pigmented targets, as we do here, or by using staining or dye-labeling techniques. During laser exposure, absorption and radiationless deexcitation convert radiant energy into heat within each target in the exposure field. The targets begin to transfer this heat to their cooler surroundings mainly by thermal diffusion, but this process takes some time and heat is initially confined

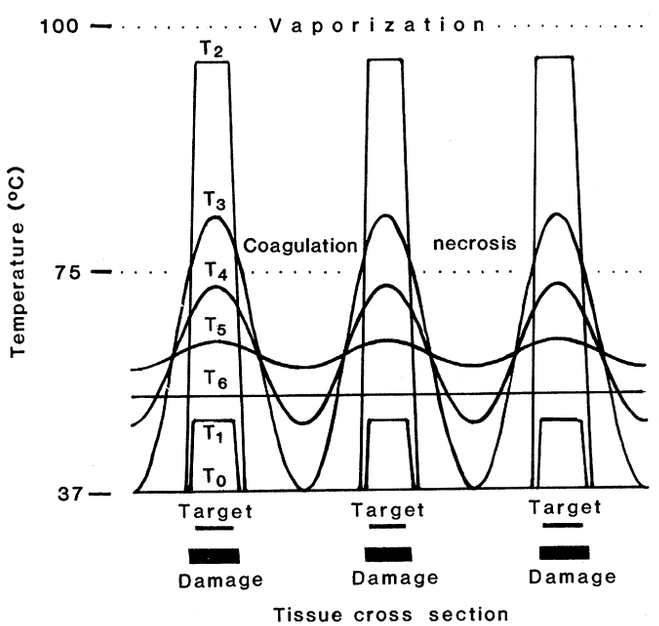

Fig. 1. Schematic temperature profiles during selective photothermolysis: T_0, before laser exposure (uniform body temperature); T_1, during laser exposure (selective rapid target heating); T_2, at the end of laser exposure (targets irreversibly damaged); T_3, one thermal relaxation time after laser pulse (targets cooling, surrounding tissue warming); T_4, two thermal relaxation times after laser pulse; T_5, five thermal relaxation times after laser pulse; and T_6, tissue slowly returing to ambient thermal equilibrium.