OTR8 - Multiple antigen immunization: a throughput platform for monoclonal antibody generation.

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Introduction:

For several decades, mouse monoclonal antibodies (mAb) were isolated using the hybridoma technology. This technique has produced numerous antibodies with high-specificity and affinity of antigen recognition over the years but it has the disadvantages of requiring high antigen quantities and being time-consuming. Multiple antigen immunization is a throughput technique described for its increased speed of mAb generation in order to attend the increased demand for antibodies in research.

Objective:

To develop a throughput method for mAb generation, focusing on reduced development time and reduced amount of antigen.

Methodology:

Four purified proteins - VP1 Hepatite A (VP1;Abcam), Hepatite B surface antigen (HBsAg; USBiologicals), dengue NS1 (NS1;ProSpec) and dengue ST2 (ST2;ProSpec) - were mixed together (10 μg each) and emulsified in Freund's complete adjuvant before intraperitoneal (i.p.) injection into female 6 week Balb/C mice. After 7 and 14 days of first immunization, mice were boosted (i.p.) with a mixture of antigens (5 μg of each) emulsified in Freund's incomplete adjuvant. Three days after booster injections, mice sera were assayed by specific ELISA for each protein individually. Twenty one days after immunization, spleen cells were homogenized and fused with SP2/0 myeloma cells (2:1), using PEG 3000-3700 (Sigma). Hybridomas were selected in HAT (hypoxanthineaminopterin-thymidine)-containing medium for 15 days and the supernatants from growing hybrids were screened by ELISA, using mixed antigens. ELISA positive wells with optic density (O.D.), at 450 nm, twice as the blank wells were considered reactive. Selected polyclonal hybridomas were grown and subcloned by limiting dilution. Each monoclonal hybridoma was assayed by specific ELISA against individual antigen. The po-

sitive monoclonal hybridomas against each protein were identified and were cryopreserved using DMSO 7.5%/SFB 42.5%/DMEM50%.

Results:

Mice pre-immune/immunized serum O.D. values after the first booster injection were VP1: 0.029/0.029; HBsAg: 0.018/0.048; NS1: 0.025/0.047; ST2: 0.024/0.120. O.D. values for the second boosting were VP1: 0.010/0.045; HBsAg: 0.010/0.325; NS1: 0.000/0.421; ST2: 0.002/0.684. After fusion and HAT selection, 185 polyclonal hybridomas were grown and the best 30 clones with O.D.> 0.5 were selected for cellular expansion. Nineteen clones were expanded in 24/6-well plates. When the polyclonals were re-tested individually in specific ELISA, we found 1 clone mono-specific for VP1 (EG7), 1 clone mono-specific for HBsAg (IB10), 1 clone that reacted against both VP1 and HBsAg (IE8), and 2 clones that reacted against both NS1 and ST2 (IF7 and GE8). Interestingly, after subcloning, we found a monoclonal hybridoma bi-especific (GE8/JB2), which reacted against NS1 and ST2, and a monoclonal hybridoma mono-specific that recognizes HBsAg (IE8/CB11).

Conclusion:

Multiple antigen immunization provided mAbs against different antigens in a shorter period of time and using less antigen, especially if compared to LATAM mAb development standard protocol (reduction of 53% in the immunization period and 82% in antigen mass; data not shown).

Keywords: antibody, immunization, multiple antigens.