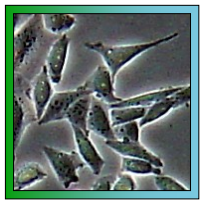

Performance Enhancing Synergy Between a Wheat Hydrolysate and Recombinant Human Serum Albumin in SP2/0 Hybridoma Cells

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Introduction

In an effort to mitigate potential risks associated with the introduction of adventitious agents from animal-derived medium components, the biopharmaceutical industry has largely shifted to the use of serum-free and/or chemically defined media in their cell culture production systems. The development of these new media has often been hindered by the inability to achieve comparable performance as is seen with cells grown in media containing Fetal Bovine Serum or animal-derived protein hydrolysates.

A variety of components may be employed to compensate for the absence of FBS or animal-derived peptones in these formulations.They may be used either as integral components of newly developed serum-free media, or as performance enhancing supplements to existing formulations, including classical media and chemically defined media. Much success has been achieved using plant-derived protein hydrolysates in combination with other supplements such as insulin, transferrin and other growth factors normally derived from FBS or other animal sources. Many of these animal-derived proteins and growth factors are now being produced in animal-free recombinant systems.

The performance benefit provided by any medium supplement is subject to its interaction with other medium components present in the basal formulation, as well as any additional supplements being employed. In some instances, a combination of supplements may provide better performance than is seen when supplementing with the individual entities. Here we demonstrate such a synergistic reaction between a wheat hydrolysate and recombinant human serum albumin used to supplement a chemically defined growth medium for SP2/0 hybridoma cells.

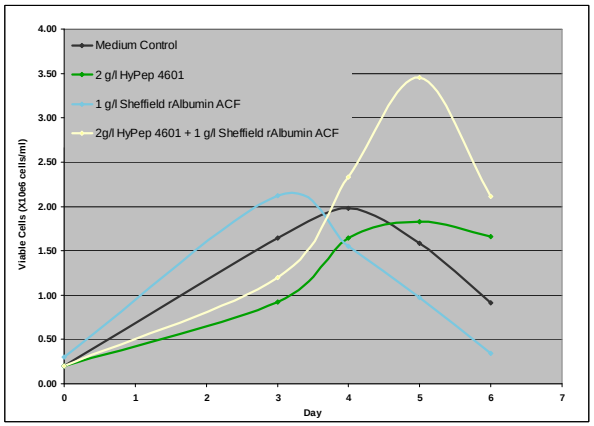


Figure 1. Growth curves for SP2/0 cells in chemically defined medium.

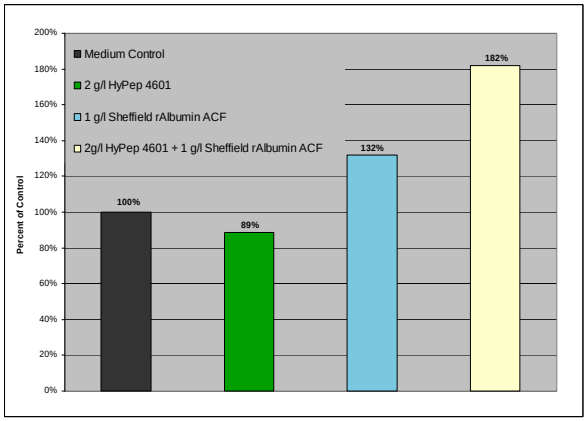


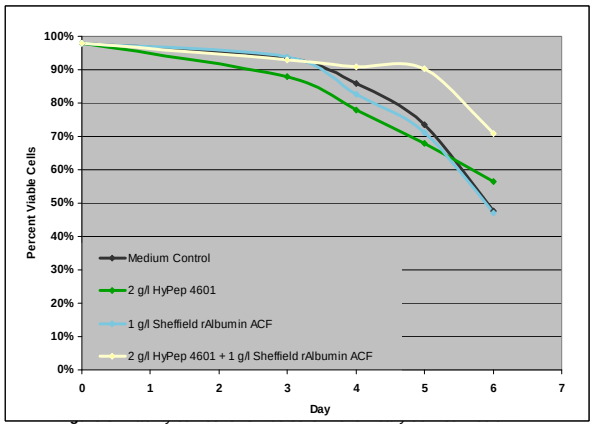
Figure 2. IgG titers for SP2/0 cells in chemically defined medium.

Materials and Methods

The SP2/0 murine hybridoma cell line was obtained from ATCC (CRL-1753) and adapted to grow in a commercially available serum-free chemically defined medium. Cultures were grown in 125 ml shake-flasks containing a final medium volume of 30 ml. Triplicate cultures were seeded at $2x10^5$ cells/ml, and incubated at 37°C in 5% CO2 at 130 rpm for 9 days. Hydrolysate and recombinant human serum albumin supplementation was achieved via the use of filter-sterilized 100 g/l stock solutions prepared in the basal medium.

At days 3, 4, 5 and 6, 400 µl of the culture supernatants were removed for assessing cell counts and viability. Cells were counted using a NucleoCounter fluorescence-based automated cell counter. At Day 9, 500 µl of the culture supernatants were removed for IgG analysis. Levels of IgG in the supernatants were measured using Protein G affinity chromatography on a Waters Model 2695 separations module equipped with a Waters Model 2487 dual-wavelength absorbance detector.

Cultures were supplemented with Sheffield™ HyPep 4601, a wheat hydrolysate, Sheffield™ rAlbumin ACF recombinant human serum albumin, and a combination of the two. Optimum dosages for either supplement were determined in earlier experiments.



Summary

Subject to the supplementation scheme, the growth curves for each of the treatments were shifted as compared with the medium control. In cultures supplemented with recombinant human albumin alone, the growth curve peaks earlier and at a higher cell density than the medium control. Growth for the wheat hydrolysate supplemented cultures was more protracted and the maximum cell density fell short of the medium control, however growth declined at a slower rate after the peak cell density was reached. The growth curve for the cultures supplemented with both wheat hydrolysate and recombinant human albumin peaked two days later, and at a significantly higher cell density than the medium control.

Neither the wheat hydrolysate nor the recombinant human serum albumin improved cell viability individually, however in combination, culture life was significantly extended over that of the medium control.

Supplementation with a combination of wheat hydrolysate and recombinant human serum albumin yielded a significantly higher IgG titer than the other treatments. While there was a more than 30% increase in IgG titer in the cultures supplemented with recombinant human albumin alone, the IgG titer in the wheat hydrolysate supplemented cultures fell short of that for the medium control. This lower titer for the wheat hydrolysate supplemented culture was likely due to the low cell densities seen during the course of the experiment.