Antibody Isotype and Productivity

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INTRODUCTION

High level antibody expression in mammalian cell lines results from a combination of factors including the choice of expression system, host cell line and process. Although the choice of antibody isotype is most likely to be determined by the effector function required for the antibody product, there are anecdotal reports that the choice of antibody isotype should be a consideration when attempting to maximise product yield.

Antibody constant regions exist as a range of isotypes. The different isotypes contribute to varying biological activity for antibodies of different isotype. For example, in the case of the four subclasses of Immunoglobulin G (IgG) antibodies, each differs in its ability to induce antibody dependent cell-mediated cytotoxicity (ADCC) as well as activation of complement [1]. IgG_1 antibodies are the most effective in ADCC, whereas antibodies of the IgG_4 subtype are considered as 'blocking' antibodies where no effector function is required. IgG_2 antibodies are considered to be an alternative to IgG_4 but are more effective in complement-dependent lysis at high antibody concentrations.

This study looked to support, or otherwise, the assumption that the choice of isotype may contribute to product concentration, by comparing productivities of cell line panels generated containing different isotypes of a model antibody. Expression vectors were constructed encoding a model antibody containing either an IgG_1, IgG_2 or IgG_4 heavy chain along with a kappa light chain. The antibody concentration achieved from 100 stable cell lines cultured in 24-well plates was then determined by Protein A HPLC. A fed-batch shake flask process, designed to mimic laboratory scale bioreactor cultures, was used to further characterise the effect of IgG_1 or IgG_4 antibody isotype on product yield in suspension culture.

MATERIALS AND METHODS

Vector construction

- GS (Glutamine Synthetase) expression vectors (Lonza Biologics, GS Gene Expression System™) encoding a model antibody containing either an IgG_1, IgG_2 or IgG_4 heavy chain gene along with a kappa light chain gene were generated. The features of the vectors are shown in Figure 1.

Stable Transfection: 24 well plate assessment

- CHOK1SV cells were transfected with the IgG_1, IgG_2 or IgG_4 vector constructs using standard electroporation methods. Transfected cells were plated out across 96-well plates. The following day, selective chemically-defined, animal component-free medium containing methionine sulphoximine (MSX) was added such that the final concentration in each well was 50 μM MSX.

- Plates were screened for developing colonies at 3-4 weeks post-transfection. For each transfected vector, 100 colonies were randomly selected and transferred to 24-well plates in medium containing 25 μM MSX. Cultures were allowed to 'overgrow' for 2 weeks, after which the cell culture medium from each well was collected and analysed by Protein A HPLC to determine the antibody concentration.

Stable Transfection: Fed-batch shake-flask cultures

- CHOK1SV cells were transfected with the IgG_1,or IgG_4 vector constructs using standard electroporation methods. Transfected cells were plated out across 96-well plates. The following day, selective chemically-defined, animal component-free medium containing methionine sulphoximine (MSX) was added such that the final concentration in each well was 50 μM MSX.

- For each vector, the productivity of 200-300 colonies in 96-well plates was assessed by ELISA and the top 150 producers transferred to 24-well plates. Following productivity assessment in 24 well-plates as above, the 60 cell lines producing the highest concentrations of antibody were transferred to T25 flasks before being adapted to suspension growth in shake flask culture.

- When acceptable and reproducible growth in suspension had been established, a culture of each cell line was prepared for fed-batch shake flask evaluation. Cells were grown for 15 days, with a nutrient feed added on day 3 and a glucose feed on days 8 and 11.

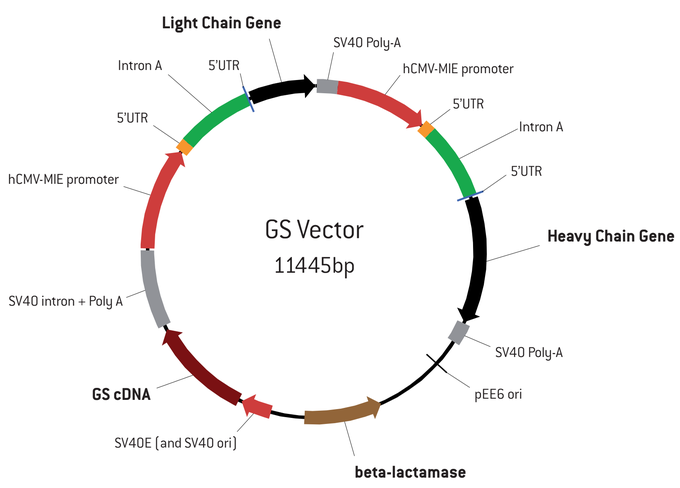
- After 15 days, culture supernatants were harvested and analysed by Protein A HPLC to determine the antibody concentration.

Data analysis

- Mean antibody concentrations were compared by ANOVA and Tukey's method for pairwise comparison of all individual means. A p value of ≤ 0.05 was considered significant.

- These experiments were designed with statistical power to detect either a 10% or 5% change in mean antibody concentration in the 24-well plate or fed-batch shake-flask study respectively.

Figure 1: Representation of the expression vector used for isotype evaluation. Lonza's GS Gene Expression System™ uses a single vector encoding both the heavy and light chain antibody genes, with expression of each chain driven by the strong human cytomegalovirus major intermediate early promoter. The GS gene is driven by the weaker SV40 promoter which is thought to bias for selection of integration into transcriptionally active sites in the genome.



RESULTS:

24 WELL-PLATE ASSESSMENT OF ANTIBODY PRODUCTION

- Cell lines were created using vectors containing an antibody IgG_1, IgG_2 or IgG_4 heavy chain constant region. Cell lines were randomly selected to sample from the entire population of the transfected pool of cells. Selected cell lines were cultured in 24-well plates for 14 days and the antibody concentration determined.

- Results were analysed by ANOVA and Tukey's post-hoc analysis (Table 1). A p value of ≤ 0.05 was considered significant.

- The mean antibody concentration produced by cell lines expressing the IgG_1 or IgG_4 molecule was 27.7 mg/L and 25.9 mg/L respectively (Figure 2). These values are not significantly different (p=0.37).

- The mean antibody concentration from cell lines expressing the IgG_2 molecule was 16.9 mg/L which is significantly different to the yields with both IgG_1 and IgG_4 (p<0.001).

Figure 2: The mean antibody concentration in medium from CHOK1SV cells stably transfected with vectors containing an IgG_1, IgG_2 or IgG_4 heavy chain construct. Box plots show the distribution of antibody concentrations in culture supernatant derived from 100 stable cell lines per vector. * indicates a statistically significant difference (p<0.001).

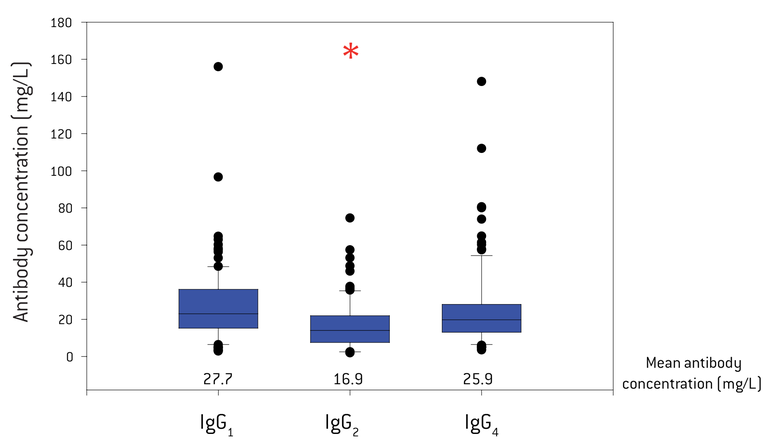


Table 1: Statistical analysis of 24-well plate data generated during stable cell line construction. n=100 per construct. Results were analysed using ANOVA and Tukey's post-hoc analysis.

	IgG_1	IgG_2	IgG_4
Mean Ab conc (mg/L)	27.7	16.9	25.9
vs IgG_1	-	-	-
vs IgG_2	p<0.001	-	-
vs IgG_4	p=0.37	p<0.001	-

RESULTS:

FED-BATCH SHAKE-ASSESSMENT OF ANTIBODY PRODUCTION

- Vectors were constructed containing an IgG_1 or IgG_4 heavy chain constant region.

- The top 150 cell lines from the transfected pool of cells were selected on the basis of productivity in 96-well plates. These cell lines were then cultured in 24-well plates and antibody concentrations determined (Figure 3).

- The mean antibody concentration expressed by cells containing the IgG_1 or IgG_4 molecule was 178.6 mg/L and 125.8 mg/L respectively (p<0.001). The result is different from that described in Figure 2. In Figure 2, the data were obtained by random sampling of an entire population of transfectants. In Figure 3, the data were obtained using a subpopulation of a second transfectant population, selected on the basis of being highly ranked for productivity.

- Fed-batch shake-flask cultures of the 60 highest producing cell lines were harvested after 15 days and supernatant samples analysed by Protein A HPLC to determine the antibody concentration.

- The mean antibody concentration from 60 cell lines expressing either the IgG_1 or IgG_4 molecule were compared (Figure 4) and results were analysed using ANOVA and Tukey's post hoc analysis.

- The mean antibody concentration expressed by cells containing the IgG_1 or IgG_4 molecule was 1286 mg/L and 1235 mg/L respectively. These values are not significantly different (p=0.37), and are at variance with the data seen in Figure 3. The cell lines taken into this screening round are a subset of those used for the experiment described in Figure 3 and are chosen based on their ranked position after analysis of the 24-well plate data.

- The antibody concentration achieved by the lead cell line for each isotype was equivalent (2428 mg/L and 2404 mg/L for the IgG_1 and IgG_4 molecule respectively).

Figure3: The mean antibody concentration in medium from CHOK1SV cells stably transfected with vectors containing an IgG_1,or IgG_4 heavy chain construct. Box plots show the distribution of antibody concentrations in 24-well plates derived from the top 150 stable cell lines selected per vector (p<0.001).

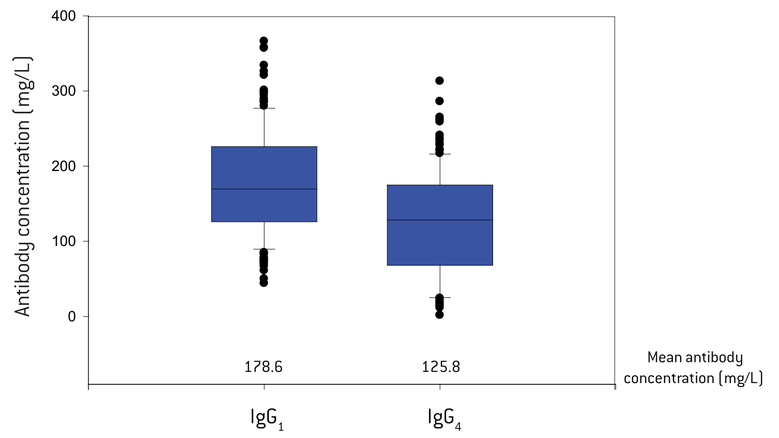
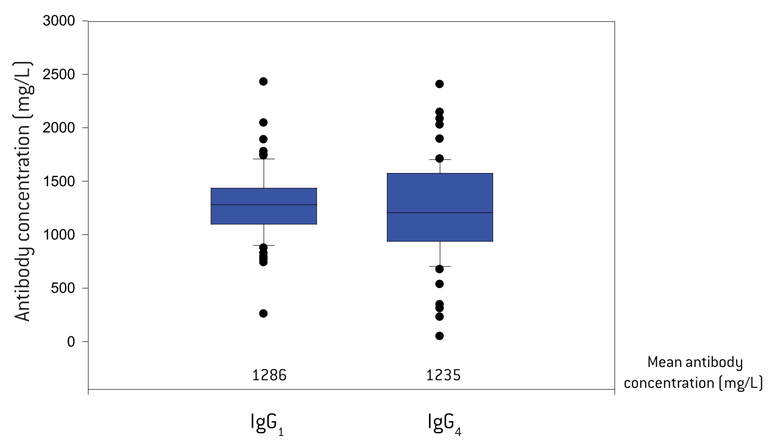


Figure 4: The mean antibody concentration in medium from CHOK1SV cells stably transfected with vectors containing an IgG_1, or IgG_4 heavy chain construct. Box plots show the distribution of antibody concentrations in fed-batch shake-flask culture supernatant derived from 60 stable cell lines per vector (p=0.37).



CONCLUSIONS

- For the model antibody used in this study, similar distributions of antibody concentrations are achievable with an IgG_1 or IgG_4 heavy chain when populations of transfectants are studied. As numbers of transfectants are reduced by selection of the higher producers, differences may be seen. In the key screening stage, a shake-flask model of the fed-batch bioreactor process, no evidence of difference between IgG_1 and IgG_4 was obtained.

- The choice between IgG_1 and IgG_4 constant regions need not be a consideration when attempting to maximise productivity. Instead, choice should rather be defined by the effector function required for the antibody product.

- For IgG_2 heavy chains, lower concentrations were seen in 24-well plates indicating that the choice of antibody isotype may affect product concentration. This may be a consideration when choosing between IgG_2 and IgG_4 isotypes. However, further work is needed to confirm the observation with IgG_2 antibodies.

REFERENCES

1. Bindon, C.I., Hale, G., Brüggemann, M. & Waldmann, H. J. Exp. Med.1988 Jul;168:127-42

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