Corning® 88-551-CM Lymphocyte Serum-free Medium for Activation and Expansion Culture of Human T Cells

Application Note

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Introduction

Corning 88-551-CM Medium was developed to support the growth of T cells activated by immobilized anti-CD3 antibody. Interleukin-2, interferon gamma, and human autologous plasma are required for Cytokine-induced Killer (CIK) induction. In this study, we compared the performance of Corning 88-551-CM medium with the counterpart product from a competitor.

Material and Methods

Preparation of peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated from fresh whole blood using the lymphocyte separation medium (LSM) (Corning Cat. No.25-072-CI) according to the supplier provided protocol. The obtained PBMCs were washed three times with PBS (Corning Cat. No. 21-040-CV, without calcium and magnesium). First wash: 1,500 rpm for 10 minutes at room temperature; second wash: 1,200 rpm for 10 minutes at room temperature; and third wash: 1,000 rpm for 10 minutes at room temperature.

Culture of cytokine-induced killer (CIK) cells

The density of PBMCs was adjusted to 2 x 10^6 cells/mL, and 5 mL of the cell suspension was seeded into a T-25 tissue culture-treated flask (Corning Cat. No. 430639). Next, 1,000 U/mL interferon (IFN)-gamma (Peprotech Cat. No. 300-02) and 5% autologous plasma were added into the medium, and the mixture was incubated at 37°C and 5% CO_2 in a humidified incubator. After 24 hours, the culture medium was supplemented with interleukin (IL)-2 (Corning Cat. No. 354043) and anti-CD3 antibody (OKT3, provided by Kohjin) at a final concentration of 300 IU/mL and 50 ng/mL, respectively. The cells were continuously cultured for 15 days before harvest. In the first 7 days, the autologous plasma level was maintained at 5%. Fresh medium containing IL-2 (300 IU/mL), but without human autologous plasma, was added when necessary to ensure that the nutrient level was sufficient to sustain cell prolifertion. The cell density was adjusted to 1 x 10^6 cells/mL each time when adding fresh media. The cells were cultured at 37°C in a humidified atmosphere of 5% CO_2 for 15 days.

Immunophenotyping

CIK samples were collected on day 10 for immunophenotyping analysis by flow cytometry. Two special double-positive markers, $CD3^+/CD8^+$ and $CD3^+/CD56^+$, were used to evaluate the surface marker expression of the expanded cells. After washing, 1 x 10^6 CIK cells were resuspended in 100 µL PBS and incubated with 10 µg/mL antibody or corresponding isotype control for 15 minutes at room temperature (light exposure should be avoided during incubation). After incubation, the harvested cells were briefly washed with PBS buffer and then resuspended in 300 µL PBS buffer for flow cytometry analysis using the BD Accuri™ C6 flow cytometer (BD Biosciences). In this analysis, 10,000 events were recorded for each sample and analyzed by histogram overlay subtraction analysis using the BD Accuri C6 software (BD Biosciences).

Results

CIK cells formed clusters on Day 5 in both Corning 88-551-CM medium and competitor medium, however, the cluster size in 88-551-CM medium was bigger than competitor medium (Figure 1). As to the cell expansion capacity, 88-551-CM medium showed an obvious advantage compared to the competitor medium from Day 7. Moreover, the cell yield finally obtained on Day 15 was 52.6% higher than that obtained with the competitor medium (Figure 2). High cell viability (>90%) was maintained during the entire experimental period for both groups (Table 1). Expression level of the specific surface markers on Day 10 was analyzed by flow cytometry analysis (Table 2).

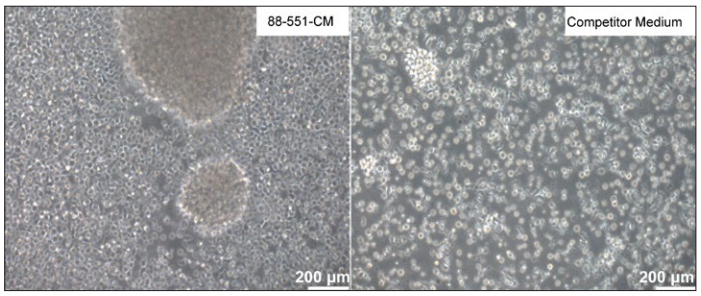

Figure 1. Cell morphology in each medium on Day 5. Greater cell clusters can be observed in Corning 88-551-CM medium than the competitor medium.

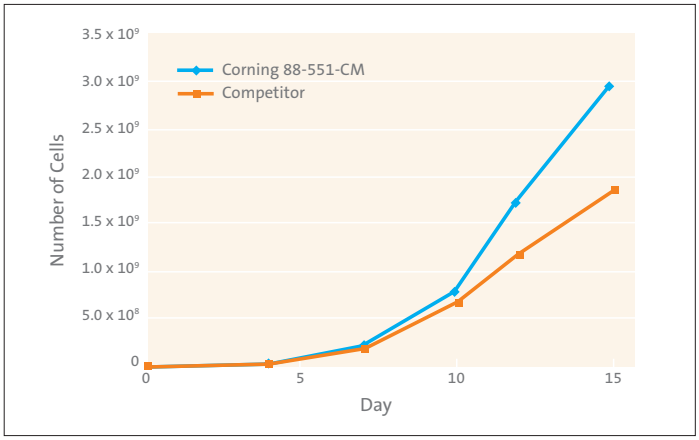

Figure 2. CIK cell proliferation capacity benchmark study when using Corning 88-551-CM medium and competitor medium.

Table 1. Cell viability statistics throughout the experimental period

Day	88-551-CM Medium (%)	Competitor Medium (%)
Day 0	95	95
Day 4	91	95
Day 7	94	94
Day 10	94	94
Day 12	92	88
Day 15	93	93

Table 2. Surface marker expression determined by flow cytometry on Day 10

Item	88-551-CM Medium (%)	Competitor Medium (%)
$CD3^-/CD8^-$	2.5	11.0
$CD3^+/CD8^-$	33.9	24.2
$CD3^-/CD8^+$	0.7	0.9
$CD3^+/CD8^+$	62.9	63.2
$CD3^-/CD56^-$	5.3	7.4
$CD3^+/CD56^-$	88.3	72.4
$CD3^-/CD56^+$	0.9	8.9
$CD3^+/CD56^+$	5.5	11.3

Conclusion

CIK cells cultured in Corning® 88-551-CM medium showed stronger proliferation capacity than cells cultured in the competitor medium, and the final harvested cell yield was 52.6% higher than the competitor medium. As to the special marker expression level on the cell surface, CIK cells cultured in Corning 88-551-CM medium showed lower expression of $CD3^+/CD56^+$ than competitor medium.

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