Asian Pacific Journal of Allergy and Immunology (1988) 6 : 3-6

ORIGINAL ARTICLES

The Influence of Microtiter Plates from Different Suppliers on Lymphocyte Proliferation

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SUMMARY **Microtiter plates have been popularly used for lymphocyte culture, but the influence of culture plates from different sources has not been investigated. In this study, the degree of mitogen-induced cell proliferation was investigated using six different brands of flat-bottomed plates. Lymphocytes from twelve normal donors were cultured for 96 hours with several mitogens including PHA, Con A and PWM. Spontaneous cell proliferation was slow and it did not differ significantly among the different plates. However, mitogen-induced cell proliferation showed a wide variation among the six types of plates used. The importance of selecting certain kinds of plates for specific purposes is emphasized.**

Microtiter plates have been popularly used in the past decades,[1,2] because they provide many advantages such as economy and convenience. The amount of medium needed is minute and the number of cells used ranges from as few as 5×10^3 per well to 5×10^5 per well.[3,4]

The type of microtiter plate used in lymphocyte culture is usually 8 wells by 12 wells, giving a total of 96 wells per plate. There are many brands in the market. Whether or not the degree of cell proliferation is influenced by different brands of plates used has not been reported. This consideration has been frequently neglected. Since mitogen-induced lymphocyte proliferation is routine work in an immunology lab, it is important to investigate the variation of cell growth when different brands of plates are used. In this study, six brands of microtiter plates were used. All precautions were taken to minimize technical variation during cell culture and harvesting.[5] Mitogens for T lymphocytes (phytohemagglutinin or PHA, and concanavalin A or Con A), and mitogen for B lymphocytes (pokeweed mitogen or PWM) were used in cell proliferation tests.[6]

MATERIALS AND METHODS

Materials

Six brands of microtiter culture plates with flat bottoms were used. They were: (A) Falcon, Cat. No. 3072, from Labware of Becton Dickinson and Co., Oxnard CA, USA; (B) Griener, Cat. No. 655180, from C.A. Griener and Söhne GmbH and Co., West Germany; (C) Corning, Cat. No. 25860, from Corning Glass Works, Corning, NY, USA; (D) Costar, Cat. No. 3596, from Costar Inc, Cambridge, MA, USA; (E) Flow, Cat. No. 76-032-05, from Flow Laboratories, Inc. McLean, VA, USA; and (F) Nunc, Cat. No. 1-67008, from A/S Nunclon, Denmark.

PHA, lot HA-16, was purchased from Wellcome Research Laboratories, Beckenham, England; Con A, lot 901261, from Calbiochem-Behring Corp., La Jolla, CA, USA; and PWM, lot 66F-9530, from Sigma, St Louis, MO, USA.

RPMI-1640 and fetal bovine serum were all purchased from GIBCO, Grand Island, NY, USA. The fetal bovine serum was heated to 56° C for 30 min to inactivate complement. The final culture medium

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was RPMI-1640 containing fetal bovine serum (10%),L-glutamine (2 mM),penicillin G (100 U/ml) and streptomycin (100 μg/ml). ^{3}H-thymidine (6.7 Ci/m mole) was purchased from New England Nuclear Co., Boston, MA, USA. The scintillation fluid was toluence-based Liquifluor (New England Nuclear Co.) containing PPO 4 g/l and POPOP 0.1 g/l.

Subjects

Twelve healthy donors were recruited from hospital employees, eight males and four females, aged from 20 to 40 (mean $\pm$ SD of 28 $\pm$ 7). They did not regularly take medication and had refrained from any medication for at least two weeks prior to blood donation.

Lymphocyte culture

Mononuclear leukocytes were separated from peripheral blood by Ficoll-Hypaque density gradient centrifugation as previously described.[7] The cells thus separated were 85% lymphocytes, 10-15% monocytes and less than 5% neutrophils. The monocytes were identified by nonspecific esterase stain.[8] The viability was more than 95% as judged by trypan blue dye exclusion.

Cells (1×10^5/well) were cultured in quadruplicates for 96 hours. The cells obtained from a single donor were cultured in all six brands of plates simultaneously. Culture conditions were identical for all brands of plates.

Cells were then pulsed with ^{3}H-thymidine (0.5 μCi/well) for the terminal 4 hours of culture and were harvested onto a glass fiber filter paper, using a semiautomatic cell harvester (Brandel, model M 24, Rockville, MD, USA.). Care was taken during cell harvesting to minimize technical variation.[5] Cells in each of 24 wells (two rows) were harvested at one time. The four wells of each quadruplicate were placed in separate rows so that harvesting variation, if any, would be randomly distributed in the quadruplicate wells. The coefficient of variance of cell proliferation in the quadruplicate cultures was around 0.05.

The isotope incorporation was measured by a standard toluene-based scintillation counting technique.

Statistical analysis

The Wilcoxon signed rank test was used to compare the degree of cell proliferation in cultures using different brands of plates.

RESULTS

Spontaneous proliferation

In the absence of mitogen, lymphocyte proliferation was minimal (Table 1). There was no difference among different brands of plates.

PHA-stimulated cultures

At suboptimal concentration of PHA (0.1 μg/ml), cells cultured in plates of brands A, B and C had better proliferation than cells cultured in other brands, and the differences were statistically significant ($p < 0.04$) (Table 1).

At optimal concentration of PHA, 1 μg/ml, cell proliferation was 4 to 5-fold better than that at 0.1 μg/ml. The cell growth in plate A was significantly better ($p < 0.05$) than it was in other plates except for plate B. The cell growth in

Table 1 Lymphocyte proliferation under various conditions

Condition	Proliferation (cpm)* in different plates						p values
	A	B	C	D	E	F	
Unstimulated	214 ±31	212 ±33	188 ±21	181 ±27	186 ±17	197 ±17	$p > 0.05$
PHA							
0.1 μg/ml	11,192 ± 2,012	10,101 ± 1,948	10,343 ± 1,794	9,618 ± 1,898	7,607 ± 1,146	8,320 ± 1,425	$p < 0.005$ (A vs E, A vs F, C vs E) $p < 0.05$ (B vs E, B vs F, C vs F)
1.0 μg/ml	49,255 ± 4,877	47,379 ±4,523	46,233 ± 5,445	45,127 ± 4,484	43,374 ±4,519	41,523 ± 4,956	$p < 0.005$ (A vs D, A vs E, A vs F, B vs E, B vs F); $p < 0.05$ (A vs C, C vs E, C vs F, D vs F)
Con A 0.9 μg/ml	8,148 ± 1,050	6,820 ± 816	7,004 ±772	6,962 ±849	6,140 ±816	6,254 ±878	$p < 0.005$ (A vs B, A vs E) $p < 0.05$ (A vs C, A vs D, A vs F, C vs E, D vs E)
9.0 μg/ml	32,872 ± 4,646	30,625 ±4,731	29,010 ± 3,653	29,444 ± 4,265	27,868 ±4,825	26,874 ± 4,847	$p < 0.005$ (A vs E, A vs F) $p < 0.05$ (A vs B, A vs C, A vs D, B vs E, B vs F)
PWM 0.1 μg/ml	5,515 ± 753	4,742 ±748	4,557 ±695	4,872 ±811	4,393 ±663	4,388 ±680	$p < 0.005$ (A vs C, A vs E) $p < 0.01$ (A vs B, A vs F)

* The numbers represent means ± SE from 12 donors

plate F was significantly less ($p < 0.05$) than that in other plates except for plate E.

Con A-stimulated cultures

At suboptimal concentrations of Con A (0.9 μg/ml), cell growth in plate A was significantly better ($p < 0.04$) than the other plates (Table 1). Cell growth in plate E was significantly less ($p < 0.03$) than that in other plates except for plates B and F.

At optimal concentration of 9.0 μg/ml Con A, cell growth in plate A was significantly better ($p < 0.05$) than that in other brands. The cell growth in plate B was significantly better ($P = 0.028$) than that in plates E and F.

PWM-stimulated cultures

At optimal PWM concentration of 0.1 μg/ml, cell growth in plate A was significantly better ($p < 0.01$) than that in other plates except for plate D (Table 1).

DISCUSSION

This study clearly demonstrates that there is a wide range of variation in proliferation when lymphocytes are cultured in plates from different sources. Plates of certain brands consistently gave better cell proliferation than the others. It was not our purpose to emphasize which brands were superior to others. There are many uses for microtiter plates, including mitogen-induced and antigen-induced cell proliferation, mixed leukocyte culture, monoclonal cell separation, and various immunoassays. In this study, we investigated only mitogen-induced lymphocyte proliferation and showed that there was a big variation in results among the plates of different suppliers. Variation with other uses of microtiter plates was not investigated, and the variation obtained in this study may not be found with other uses. Also, it is possible that plates of various brands may be superior in certain biological experiments.

Throughout the study, optimal conditions for cell proliferation were adopted. First, flat bottom culture wells were used as these were previously reported to provide much better cell proliferation than conical or round-bottom wells.[7,9] Second, 96-hr cultures provided better cell proliferation than other timings.[10] Third, optimal concentrations for PHA, Con A and PWM, and suboptimal concentrations of PHA and Con A were used for cell stimulation.[10] Fourth, the whole population of mononuclear cells obtained by Ficoll-Hypaque density gradient centrifugation was used, instead of lymphocytes alone. The monocytes contained in the mononuclear population are essential for lymphocyte proliferation.[11,12]

Care was taken, in the following ways, to lower experimental variations. 1) Healthy blood donors were selected with a narrow age range of 20 to 40 years (mean ± SD, 28 ± 7). 2) Blood was all drawn at 9 AM. 3) No medication had been taken by the donors for at least two weeks prior to the experiment. 4) Since there is a wide range of individual variation, as many as twelve donors participated in this study. 5) Care was taken in cell harvesting as mentioned in Materials and Methods.

Plates of brand A always gave the best cell proliferation, and the difference was more pronounced at suboptimal mitogen concentrations. For example, plate A had 26% higher cell proliferation than plate F at PHA 0.1 μg/ml, and only 16% higher proliferation than plate F at PHA 1.0 μg/ml. This phenomenon also occurred in Con A cultures and among plates from various sources. Therefore, in any experiments with low mitogenicity, the selection of an optimal plate for lymphocyte culture appears to be crucial. It is not clear whether or not this is also true for antigen-induced lymphocyte proliferation which provides a weaker mitogenic stimulation. This deserves further investigation.

The reason why plates from different sources result in different degrees of cell proliferation is not clear. All plates were made of polystyrene. The cultures using different plates were carried out at the same time, under the same conditions, and by the same technician using exactly the same procedures. There are two possibilities that may account for the culture variation. 1) Differences in the fitness of plate lids may have caused differences in ventillation that resulted in a change of pH in the culture media. 2) There may have been some minor degree of variation in plate composition or coating material(s) not indicated in the accompanying literature.

ACKNOWLEDGEMENTS

This study was supported in part by grant NSC 76-0412-B010-40 from the National Science Council, ROC, and also by the Clinical Research Center, Institute of Biomedical Sciences, Academia Sinica.

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