

Activation of Human Splenic B Lymphocytes by an Anti - Human IgM Antibody

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İNSAN DALAK B LENFOSİTLERİNİN
ANTI-HUMAN IgM ANTİKORLARI İLE
AKTİVASYONU

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SUMMARY

This study describes the triggering of human splenic B lymphocytes using a monoclonal anti-human IgM antibody. In addition, assay systems for B Cell Growth Factor (BCGF) and B Cell Differentiation Factor (BCDF) were established employing MLR supernatant as T cell-originated soluble factor.

Key Worth: Lymphocyte Aktivation Anti-Human IgM

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ÖZET

Bu çalışmada insan dalak B lenfositlerinin monoklonal anti-human IgM kullanılarak stimüle edilişi gösterilmiştir. Karma lenfosit kültürü (MLR) süpernatandan T lenfosit faktörü olarak yararlanılarak B hücresi gelişme faktörü (BCGF) ve B hücresi farklılaşma faktörü (BCDF) çalışmaları gerçekleştirilmiş ve sonuçları sunulmuştur.

Anahtar Kelimeler: B Lenfosit Aktivasyonu Anti-Human IgM.

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For the first time in 1965, Sell and Gell demonstrated that anti-immunoglobulin antibody could induce blastogenesis of rabbit lymphocytes (1). This finding led to further investigations to reveal the triggering mechanisms- of B cell activation. The discovery of presence of membrane and S on B lymphocytes and studies carried out employing anti-ju showed human lymphocytes can be activated by this way (2, 3). Romagnani, in 1981, demonstrated SAC (Staphylococcus aureus, Cowan strain I) is also capable of stimulating lymphocytes by binding to surface immunoglobulin (4, 5). Low levels of anti-Ig treatment enable cells to pass into G₁ phase of the cell cycle from the resting G₀ state with an increase in the size and RNA content of the cells. However, this does not allow the stimulated cell to enter S phase. Higher concentrations of anti-Ig is necessary for cells to progress into S phase (6, 7). Nevertheless soluble factors secreted by activated T cells are required for activated B cells to further progress into antibody forming cells (7,8).

This paper describes a system which allows us to study assays of B cell growth factor (BCGF) and B cell differentiation factor (BCDF). For this aim a

set of monoclonal anti-human IgM antibodies was produced and employed for triggering B lymphocytes.

MATERIALS AND METHODS

Animals: Female Balb/C mice, 60-10 week-old obtained from Imperial Cancer Research Fund, were used as a source of parental immune spleen cells.

Production of monoclonal antibodies: Mice were injected with 100 µg human IgM. Human IgM was purified from a serum of a patient with Macroglobulinemia. Fusions were carried out 3 days after boosting the animals i.v. In short, 10⁸ spleen cells from the immunized mice were fused using 50% polyethylene glycol with 5 x 10⁷ Sp 2/0 nonproducer myeloma cells. Irradiated (2000 rad.) Balb/c spleen cells were employed as the feeder layer. Hybrids were grown in 24-well plates (20 plates) in Iscove's modified Dulbecco's medium (IMDM) supplemented with ten per cent heat inactivated fetal calf serum and hypoxanthine-aminopterin-thymidine (HAT).

Cells: Twice-rosetted splenic B cells (human spleen cells were obtained from a person who under-

went splenectomy due to a traffic accident, frozen and kept in liquid nitrogen) were used in proliferation and differentiation experiments. Cultures were incubated in 100% humidity, and 5% CO₂ in air at 37°C. A series of different monoclonal anti-human IgM antibodies were employed to stimulate B cells. As a positive control a monoclonal anti-human IgM antibody was used (Cappel Laboratories, Cochranville PA). The medium (IMDM) served as the negative control.

Proliferation assay: The proliferative response of cultures was estimated after 72 hours by adding 0,5 µCi of ¹²⁵I dUrd (Amersham). Cells (10³ to 10⁶ cells in 200 µl) are allowed 6 hours for ¹²⁵I dUrd incorporation and then transferred to filters (Whatman) and washed extensively with water. Filters were counted in a gamma counter. The arithmetic mean of triplicate cultures is reported as cpm per culture.

Measurement of secreted Ig in culture fluids: To test antibody formation an enzyme-linked immunosorbent assay (ELISA) was used. Immunoglobulin in each culture supernatant was assayed by Elisa on day 6. Flat bottomed 96 wells microtitre plates (Linbro) was coated with rabbit anti-human IgC Fc fragment (Dako). Then standards and samples were incubated 1 hour at 37°C in a humidified atmosphere. After washing peroxidase conjugated rabbit anti-Fc was added to each well. Between all steps tween containing phosphate-citrate buffer (pH 5.0) was employed for washing. Following washing, O-phenylenediamine (Sigma) was added and the plates were read at 492 nm in a Titertek Multiskan MC photometer. The optical density values were converted to ng/ml by plotting against reference curves.

Mixed lymphocyte reaction (MLR) supernatants were used a soluble T cell factors. Those MLR supernatants obtained from human peripheral blood lymphocytes at 72 hour, were employed in cultures at different concentrations.

RESULTS

The effect of different concentrations of anti-human IgM antibody on resting human splenic B lymphocytes was shown on Figure 1. When anti Ig was used alone without any growth factor there were no evidence suggestive for lymphocytes proliferation. Similarly growth factor alone was not capable of driving the cells to S phase of cell cycle. However, when anti-Ig and MLR were used together, this time BCGF activity was demonstrated by increased ¹²⁵I dUrd uptake of the cells. Best BCGF activity in this assay system was obtained with highest anti-Ig (30% v/v) and MLR (10% v/v) concentrations employed in study and 10⁵ cells per well. Otherwise using less than 10⁵ cells per well gave no significant ¹²⁵I dUrd incorporation (Figure-2).

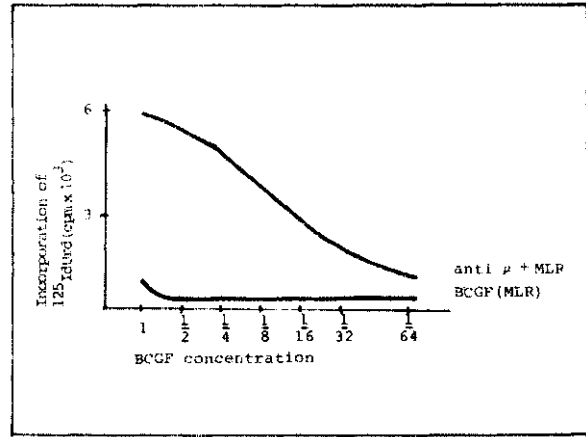


Figure-1. Relation between B cell proliferation and BCGF concentration.

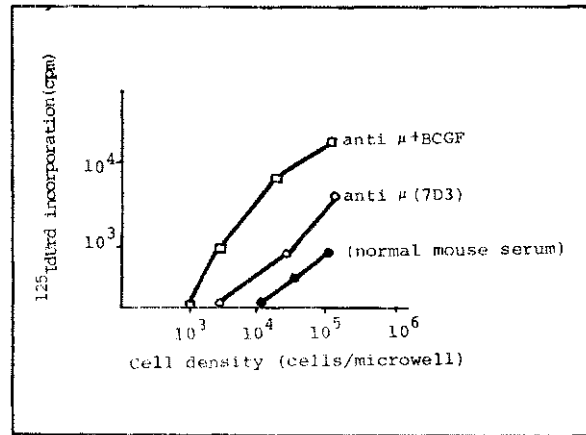


Figure-2. Relation between the magnitude of B cell proliferation and cell density.

On differentiation assay, two of our hybridoma supernatants having anti-IgM activity, together with MLR supernatant enable resting splenic B cells to produce IgG.

One of them (7D3) gave rise to even better results than cappel anti /! (Table-I) which is known to have a potent anti-human IgM activity.

DISCUSSION

This study brings further support the hypothesis both anti-Ig and soluble T cell factors are required for resting human splenic B cells to develop into antibody forming cells. Kishimoto et al for the first time showed two signals-anti Ig and lymphokines from activated T cells-drive B cells into antibody secreting cells (9, 10). It should be remembered that there are differences from one species to another species. It was shown that anti-Ig alone at high concentrations

Table -1
Immunoglobulin Secretion of Human Splenic B Lymphocytes

Stimulant	Measured Ig in Culture Supernatant (ng/ml)
Anti p. (cappel)	21
Anti p (8 Bl)	17
AntiM (7D3)	20
MLR supernatant	17
Anti p. (cappel) + MLR	225
Anti/i (8 Bl) + MLR	228
Anti p (7d3) + MLR	275

can induce proliferation in lymphocytes from humans, mice rabbits, chickens (1,11,12,13,14,15).

Our findings demonstrated that at least 10⁶ cells per well should be used to obtain a meaningful proliferation and antibody secretion. This result is in accord with previous studies (16).

The results of BCGF assay seem to be apparently unsatisfactory. Search should be made to find an-

other source which can provide with powerful activity for BCGF. T cell clones T-T cell hybridomas or supernatants from T cell tumors may well be good candidates as source of BCGF, so further studies using substances from these systems may be beneficial in 'obtaining a good proliferative response. Otherwise BCGF assay is very promising in comparison to BCGF assay. Despite weak BCGF activity of the MLR supernatant, antibody production in the BCGF assays appeared to occur at quite high levels.

In fact, our data in some respect, seem to slightly challenge with current model of B cell activation. According to the model offered by Kishimoto, BCGF and BCGF are different lymphokines showing their effects sequentially at different stages of B cell maturation (7, 9, 10). However, this study might allow us to consider that there may be a maturation factor including both BCGF and BCGF activities. This result may possibly due to weaker effect of employed MLR supernatant in terms of BCGF.

In the present study, monoclonal anti-human IgM has seemed to operate since resting B cells was prompted to antibody secretion. On the other hand neither anti-J nor SAC is in vivo physiological stimulators. In view of that elucidation of triggering mechanisms of B cell activation needs further investigations.

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