STIMULATION OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES BY A SODIUM PERIODATE ($NaIO_4$) INDUCED GROWTH FACTOR

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SUMMARY

Sodium periodate treated lymphocytes engage in cell to cell interaction and also yield a soluble growth factor that stimulates DNA synthesis when added to naive autologous cells. Adherent cells enhance the factor mediated activity. Sodium periodate stimulated lymphocytes, treated with mitomycin C, produced no growth factor activity. Partial characterization of the factor indicates that it is non-dialyzable, resistant to ribonuclease, and sensitive to heat, trypsin, and papain.

INTRODUCTION

The response of mitogen activated lymphocytes is affected by a variety of determinants that include the lymphocyte population (T cell and B cell or subtype of each), participation of monocytes or macrophages, cell to cell interactions, and the production of soluble factors that subsequently modulate or directly stimulate cell division (1-7). Sodium periodate ($NaIO_4$) is a particularly useful mitogenic agent for the study of soluble growth factors because excess $NaIO_4$ can be removed from treated cells, whereas macromolecular mitogens may contaminate the media in which stimulated lymphocytes are cultured. In this report we investigated a soluble growth factor produced by human peripheral blood lymphocytes after treatment with $NaIO_4$. We present here a partial characterization of the lymphocyte growth factor (LGF) and its effect on lymphocytes.

MATERIALS AND METHODS

Lymphocyte Cultures. Blood (500 ml) was collected from healthy donors in sterile bags containing citrate phosphate·dextrose (Fenwal). The platelets were removed and the leukocytes were separated from other blood components as

previously described (8). The isolated lymphocytes were washed 2 times with minimal essential medium (MEM), resuspended in McCoy's 5A medium (Gibco) at a concentration of 5 x 10^6 cells/ml, supplemented with 10% (V/V) autologous serum, L-glutamine, and penicillin/streptomycin, and incubated overnight in glass bottles (37°, 95% air, 5% CO_2 atmosphere). Microscopic examination of the cells demonstrated that the preparation contained greater than 90% small lymphocytes. The lymphocyte cultures were then diluted to 2 x 10^6 lymphocytes/ml in McCoy's 5A medium for the experimental studies.

Depletion and Enrichment of Lymphocyte Cell Cultures with Adherent Cells. Lymphocytes (2 x 10^6 cells/ml; 10 ml cultures) were allowed to attach to plastic culture flasks (Falcon) for 12 to 18 hours. During this period, each cell culture was gently agitated a minimum of 3 times to allow adherent cells to contact the surface of the flask. The unattached lymphocytes were then removed and used as responder cells in subsequent experiments with LGF. The addition of one-half the original volume (approximately 1.5 x 10^6 cells/ml, 5 ml) of non-adherent cells to the adherent cell monolayers constitutes the adherent cell enriched lymphocyte cultures.

Stimulation of Lymphocytes by $NaIO_4$. Lymphocytes were suspended in phosphate buffered saline (PBS) at pH 7.4 at 10^7 cells/ml and treated with $NaIO_4$. Previous studies demonstrated that $NaIO_4$ at a concentration of 5 mM for 10 minutes at 4°C produced maximal stimulation with minimal cellular damage (8, 9). Following treatment with $NaIO_4$, cells were washed 2 times with MEM to eliminate any remaining unreacted $NaIO_4$.

Preparation of LGF. Lymphocytes stimulated with $NaIO_4$ as described above were cultured for 24 hours in complete medium (10^7 cells/ml). Cells were removed by centrifugation (2000 x g, 15 min.), and the media was filtered through a 0.22 μm filter (Millipore). Alternatively, non-stimulated (2 x 10^6 cells/ml) and $NaIO_4$ treated lymphocytes (4 x 10^6 cells/ml) were washed and placed on opposite sides of a parabiotic chamber (Bellco) separated by a 0.22 μm filter.

Partial Characterization of LGF. Media containing LGF were treated with insolublized trypsin (enzite-trypsin 0.64 units/mg, 32 units/10 ml of LGF media, 4 hr., 25°C, pH 8.1), papain (enzite-papain 0.350 units/mg, 175 units/10 ml of LGF media, 4 hr., 25°C, pH 7.0), or ribonuclease (enzite-ribonuclease 0.194 units/mg, 485 units/10 ml of LGF media, 4 hr., 37°C, pH 7.0) (enzite enzymes from Miles Laboratories). The media were then centrifuged (2000 x g, 30 min.) and filter sterilized (0.2 μm filter). These media were then diluted 1 : 5 with fresh complete medium and tested for mitogenic activity on naive autologous lymphocytes.

Medium containing LGF was also heated to 100°C for 5 minutes, cooled, filter sterilized, diluted, and tested for activity.

RESULTS AND DISCUSSION

As shown in Figure 1A, the mean response of lymphocytes from 25 different donors to mitogenic stimulation occurred with the peak of DNA synthesis at about 48 hours for $NaIO_4$ and 72 hours for phytohemagglutinin (PHA). Although there was considerable variation between individual donors (approximately 3-fold), the experimental deviation between duplicate points within each pulsing period was < 10%.

Naive lymphocytes cultured in dilutions of media from $NaIO_4$ treated cells exhibited DNA synthesis with maximal activity attained at 120 hours (Fig. 1B). The mean activity was approximately 25% of the $NaIO_4$ response. Since the media that contained LGF had been centrifuged and filtered through a 0.22 μm filter, the possibility of cellular contamination was unlikely. Thus, the stimulatory activity observed was due to a soluble factor(s) liberated by $NaIO_4$ treated lymphocytes.

The presence of LGF was also demonstrated in parabiotic chambers. In these experiments (Fig. 1C), $NaIO_4$ treated cells were placed in a chamber separated from untreated lymphocytes by a millipore filter. Although the magnitude of the activity was lower, naive lymphocytes responded with a peak of activity at 120 hours, a time consistent with that observed in lymphocytes

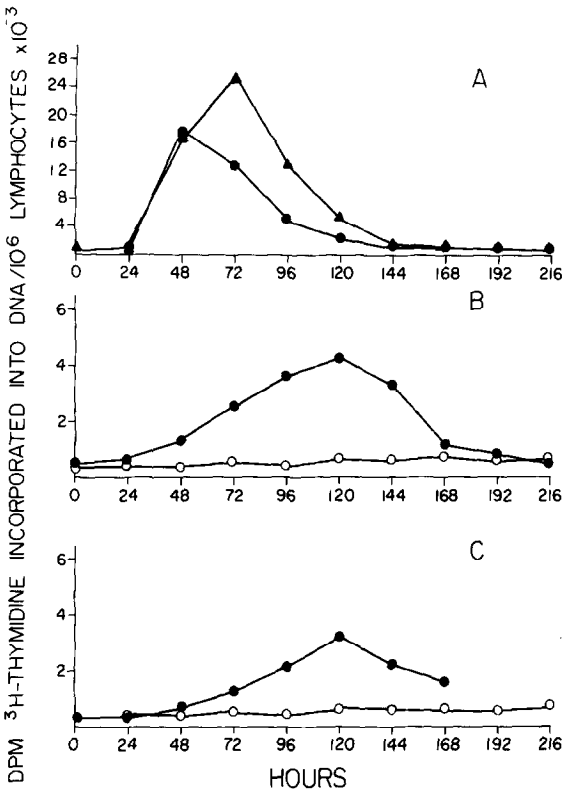

FIGURE 1A. Response of human peripheral blood lymphocytes to $NaIO_4$ (●—●) and PHA (▲—▲). At 24 hour intervals after stimulation, duplicate samples were pulse labelled with ^3H-thymidine (0.5 μCi/ml for 2 hrs at 37°C). Shown are the mean values for 25 different donors (PHA peak time of DNA synthesis, 72 hrs, mean value of 24.8 x 10^3 dpm/10^6 lymphocytes with a standard error of ± 11.3) and for 24 donors ($NaIO_4$ peak time of DNA synthesis, 48 hrs, mean value of 17.1 x 10^3 dpm/10^6 lymphocytes with a standard error of ± 8.4).

FIGURE 1B. Response of autologous lymphocytes to lymphocyte growth factor (culture flasks). The mean values shown represent experiments performed in duplicate on lymphocytes obtained from 24 different donors (●—●). Maximal synthesis occurred at about 120 hours with a value of 4.2 x 10^3 dpm/10^6 lymphocytes (standard error of ± 2.4). Untreated controls (○—○) gave a value of 0.67 x 10^3 dpm/10^6 lymphocytes with a standard error of ± 0.26.

FIGURE 1C. Response of untreated autologous lymphocytes to lymphocyte growth factor (parabiotic chambers). $NaIO_4$ treated lymphocytes are placed in one chamber separated by a 0.22 μm millipore filter from the chamber containing naive autologous lymphocytes. Shown are the mean values from 12 different donors (●—●) with a maximum at 120 hours (3.2 x 10^3 dpm/10^6 lymphocytes) with a standard error of ± 1.6. Untreated lymphocytes (○—○) gave a mean value of 0.67 x 10^3 dpm/10^6 lymphocytes with a standard error of ± 0.26.

TABLE 1

INHIBITION OF SOLUBLE GROWTH FACTOR STIMULATED LYMPHOCYTES BY MITOMYCIN C

Time in Culture (hrs)	L_N	$L_N + S_{(L_{IO_4})}$	$L_N + S_{(L_{Mit\ C + IO_4})}$
24	0.3	0.6	0.4
48	0.3	2.0	0.7
72	0.4	2.1	0.7
96	0.4	2.4	0.9
120	0.6	2.2	0.5
144	0.6	0.8	0.6
168	0.5	0.3	0.3
192	0.3	0.1	0.1

The results are expressed in dpm x $10^3/10^6$ lymphocytes as determined by pulse labelling with 0.5 μCi ^{3}H-thymidine/2 x 10^6 lymphocytes for 2 hours at the times indicated. All numbers represent a minimum of duplicate samples from at least 2 different donors. Naive lymphocytes (L_N) are cells that received no mitogen treatment.

Media $S_{(L_{IO_4})}$ or $S_{(L_{Mit\ C + IO_4})}$ from cells treated with $NaIO_4$ (for 10 minutes) or mitomycin C and $NaIO_4$ and cultured for 24 hours were added to unstimulated lymphocytes (L_N). L_N and S were diluted with fresh media to give an equivalent ratio of one stimulator cell to one responder cell.

treated directly with diluted media from $NaIO_4$ treated cells. LGF was observed in media from $NaIO_4$ stimulated lymphocytes from 24 of 29 individual donors. The reason for the absence of LGF activity in some cultures is unknown. Cultures that failed to respond to $NaIO_4$ did not produce LGF, but a few responded to $NaIO_4$ without a concomitant detection of LGF activity. These results indicate that cell to cell contact is not the sole parameter involved in the response of lymphocytes to $NaIO_4$, as has been suggested by others (10).

Table 1 shows the response of naive lymphocytes to a LGF produced in

medium TC 199. Cells that were treated with media from $NaIO_4$ treated lymphocytes demonstrated a peak of activity at 96 to 120 hours with a subsequent decrease in activity at later times. In contrast, medium from mitomycin C blocked - $NaIO_4$ treated lymphocytes yielded media that did not stimulate naive lymphocytes to undergo DNA synthesis. These results suggest that either DNA or RNA synthesis is required for the production or release of LGF. We have found little or no LGF activity produced in cultures depleted in adherent cells. In contrast to our results, O'Brien et al. (11) failed to find evidence for a soluble factor in the medium of $NaIO_4$ stimulated lymphocytes. However, these investigators cultured $NaIO_4$ stimulated lymphocytes for only 96 hours. Furthermore, we found the magnitude of LGF activity to be 2-fold greater in McCoy's 5A medium than in medium TC 199.

LGF is inactivated by treatment with either trypsin or papain, but ribonuclease does not affect its activity. Heat treatment eliminated the LGF activity. Thus, it appears that LGF is probably a protein or glycoprotein with molecular weight greater than 5,000 daltons (the exclusion limits of the dialysis membrane).

The partial depletion or enrichment of adherent cells in lymphocyte cultures results in different responses to LGF. The response is diminished when the adherent cell population is reduced, and the response is enhanced in adherent cell enriched lymphocyte cultures.

Three different mechanisms that may be involved in the response of lymphocytes to $NaIO_4$ include: a) direct stimulation of the cell; b) indirect stimulation due to cell to cell contact; and c) stimulation by soluble growth factors. Since $NaIO_4$ treated lymphocytes exhibit DNA synthesis within 48 hours as do syngeneic mixed lymphocyte cultures, it is likely that (a) and (b) above contribute to this early response. We have shown here that LGF induces DNA synthesis at a significantly later time. Whether this LGF is produced by lymphocytes stimulated with other mitogens is unknown, but factors that modulate lymphocyte activation have been demonstrated in other lymphoid systems.

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