EFFECTS OF ENVIRONMENTAL pH ON THE PRODUCTION OF HEMATOPOIETIC GROWTH FACTORS

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Abstract

Cellular and tissue activities highly depend on environmental pH. Murine lung tissue, when cultured properly *in-vitro*, is a potent producer of hematopoietic growth factors. We have studied the effect of pH on the production of hematopoietic growth factors and protein synthesis by the murine lung *in-vitro*. Various concentrations of NaHCO3 were used to adjust the pH of the culture medium under constant CO_2 partial pressure. The results indicated that hematopoietic growth factor production and protein synthesis by the lung were pH dependent. This dependency was not a result of variations in cation (Na^+) concentration in the culture medium because Na^+ reconstituted samples showed the same pattern of dependency on pH as did the unreconstituted samples. Maximum rate of protein synthesis by the lung was at pH 7. 6. However, the maximum rate of specific production of hematopoietic growth factors was observed at pH 8.

Introduction

Hematopoietic growth factors, also called colony-stimulating factors (CSF), control the proliferation and differentiation of blood forming cells. Four major types of CSF has been identified in human and murine systems. These include granulocyte-macrophage CSF (GM-CSF), granulocyte CSF (G-CSF), macrophage CSF (M-CSF), and multi CSF [1]. These are glycoproteins whose genes have been identified and cloned [2-5]. Although much detail is known about the biochemistry and molecular biology of these factors, very little is known about the cellular source and the mechanism by which the production of these factors are regulated in various tissues [6]. CSFs are produced by different tissues and cell lines in the culture. However, the lung conditioned medium (LCM) is the richest source of CSF and contains mainly GM-CSF [15].

The importance of the environmental pH in the regulation of cell function has recently received considerable attention. pH variations in the range of 6.9-8.0 profoundly modify the growth of mammalian cells in culture [7]. The optimal pH for the growth of normal and malignant cells vary between 6.8-7.9 [8]. The transformation of Syrian hamster embryo cells by benzo (a) pyrene was dependent on the pH of the medium [9]. External pH also affected the expression of various cell type specific markers, particularly mRNAs [10]. Also,

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protein synthesis is affected by pH. In a cell-free translation system a sharp increase in the rate of protein synthesis was observed when pH was increased from 6.9 to 7.4 [11].

Cells have developed efficient systems for counteracting changes in extracellular pH and thus regulating the intracellular pH (pHi). These include a Na^+/H^+ antiporter [12], and possibly several anion antiporters such as Na^+ dependent and Na^+ independent $Cl^-/HCO3^-$ antiporters [13]. The Na^+/H^+ antiporter and Na^+ dependent $Cl^-/HCO3^-$ antiporters regulate pHi on the acid side while Na^+ independent $Cl^-/HCO3$ antiporter is important in regulating the alkaline side of intracellular pH [14].

We have examined the effect of extracellular pH on the CSF production and protein synthesis by the lung tissue. The results demonstrated that the environmental pH affected the CSF production and protein synthesis. The optimal pH for protein synthesis and CSF production was determined, and a specific effect on the CSF production was demonstrated at pH 8.0.

Materials and Methods

Animals: Balb/c mice of 20-30 g were used in all experiments. Both sexes were used randomly.

CSF Production: Mice lung was finely minced and 0.5 g was cultured in 5 ml of serum free Dulbecco's Modified Eagle Medium (DMEM) supplemented with 30 mg/lit asparagine and antibiotics (200 U/ml penicillin and 200 mg/ml streptomycin) in 5 ml plastic petri-dishes (NUNC) and incubated at 37°C in 5% CO_2 for 48 hrs. The pH of the medium was adjusted with various concentrations of NaHCO3 in the culture medium.

The LCM thus prepared was centrifuged at 3000 g for 30 mins. at 4°C. The supernatant was heated at 56°C for 30 mins, centrifuged as above, and the supernatant was dialyzed against 2 changes of distilled water for 48 hrs. The dialyzed LCM was centrifuged at 3000 g for 1 hr at 4°C and the clear supernatant was used as the source of the CSF without further purification.

CSF Bioassay: The LCM was sterilized by filtration through 0.45 μm membrane filters (Millipore). Polyethylene glycol at the final concentration of 1% was added to the LCM before filtration.

The biological activity of samples were assayed by culturing 10^5 bone marrow cells of mice in 35 mm plastic petri-dishes (NUNC) containing 1 ml of DMEM (as described above) supplemented with 0.3% agar and 20% fetal calf serum in the presence of 0.1 ml (10%) of CSF. Seven days after incubation at 37°C in fully humidified atmosphere of 5% CO_2, the colonies containing more than 50 cells were scored under a dissecting microscope.

Protein Biosynthesis: Protein biosynthesis was measured by the amount of incorporation of ^{14}C labelled L-leucine (50 μ Ci/ml, Amersham) at the final concentration of 0.2 μ Ci/ml in the culture medium, into the nondialyzable fraction of the LCM. Radioactivity was measured by liquid scintillation counter (Packard).

Salt Reconstitution: The Na^+ concentration of the culture medium at various pH was measured by flame photometer. A standard concentration curve of NaCl was generated and used for calculation of Na^+ concentration from data obtained from the flame photometer. Various amounts of NaCl was added to the culture medium to obtain similar concentration ofNa^+ in samples with different pH.

Results

1- The pH of the Culture Medium: Various concentrations of NaHCO3 in the culture medium was used to obtain the desired pH under constant incubation conditions at 5% CO_2. Table 1 shows the pH values obtained for different concentrations of NaHCO3. Each point is the average of pH values of blank samples taken 3,24, and 48 hrs after incubation of the culture medium in 5% CO_2.

2- The Effect of pH on the CSF Production: The culture medium was initially incubated for at least 3 hrs. to reach the desired pH. Then the lung tissue was finely minced into this culture medium and further incubated for 48 hrs. The biological activity of these CSF samples was determined as the number of colonies they could support in the agar culture of bone marrow cells. The biological activity depends on the amount of CSF produced by the lung tissue. The results are shown in Figure 1. It can be seen that at the acidic side the CSF production increased smoothly as the pH was increased from 6.6 to 7.8. Then it decreased as the pH was further increased from 7.8 to 8.2.

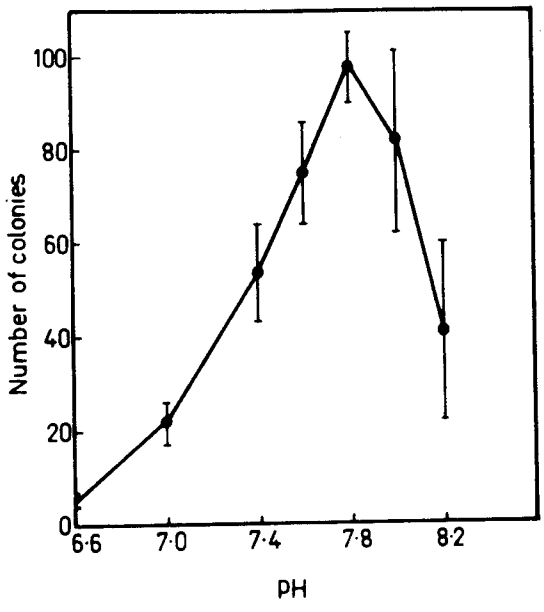

Figure 1- The effect of environmental pH on the *in-vitro* production of CSF by the lung tissue. Number of colonies stimulated by each CSF sample is plotted versus the pH of the sample. Mean ± SE of 5 experiments.

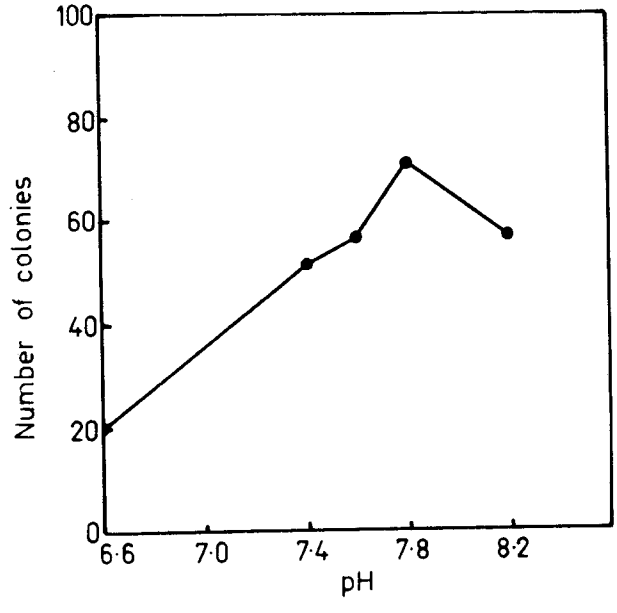

Figure 2- The effect of salt reconstitution on the pH dependence of CSF production by the lung. Mean ± SE of two experiments.

3-Effect of Salt Concentration: We studied the effect of salt concentration on the CSF production because as indicated in Table 1, increasingly higher concentrations of NaHCO3 were added to the culture medium to adjust the pH to higher values. Therefore, it was suspected that the observed effect of pH on the CSF production might be due to increasing the Na^+ concentration in the medium. To examine this possibility a series of experiments were performed in which the Na^+ concentration in the medium at all pH values was reconstituted to maintain a constant level (approximately the Na^+ concentration in the pH 8.2 sample) by adding the appropriate amount of NaCl to the culture medium. The Na^+ concentration before and after NaCl additions was measured by the flame photometer. Table 2 shows the Na^+ concentration of each sample and the amount of NaCl added to each sample. When these reconstituted mediums were used to prepare CSF, qualitatively similar results were obtained with respect to non-reconstituted samples (Figure 2). Here again, the CSF production increased as the pH was increased from 6.6 to 7.8. There was a decrease in the CSF producton

Table 1- The concentration of NaHCO3 used to obtain various pH

Desired pH	6.6	7.0	7.4	7.6	7.8	8.0	8.2
NaHCO3 mg/lit	210	482	1300	1928	2920	5427	6000
pH obtained	6.541 ± 0.08	6.95 ± 0.07	7.46 ± 0.06	7.7 ± 0.12	7.83 ± 0.07	8.07 ± 0.08	8.22 ± 0.09

1) Each point is the average of pH values of samples taken 3, 24, and 48 hrs. after incubation of the culture medium in 5% CO_2.

Table 2- The concentration of NaCl used to reconstitute the salt concentration of samples with various pH.

Desired pH	6.6	7.0	7.4	7.6	7.8	8.0	8.2
NaHCO3	216	482	1300	1928	2920	5427	6000
Na^+ concentration, mg/lit	2400	2600	2800	3100	3300	4000	4100
NaCl mg/lit	5000	4816	4248	3810	3118	1376	974
Na^+ concentration after reconstitution, mg/lit	4200	4000	4100	4100	4200	4200	4200
pH obtained after reconstitution	6.51	6.95	7.45	7.62	7.8	8.0	8.13

when the pH was increased from 7.8 to 8.2.

4- The Effect of pH on the Protein synthesis by the Lung: To further investigate the possible mechanisms involved in the effect of pH on the CSF production, we also examined the effect of pH on protein synthesis and release by the lung *in-vitro*. DMEM tissue culture medium containing ^{14}C-leucine was treated for pH adjustment as described before and was used for preparation of the LCM. The radioactivity incorporated into the LCM was counted. The results are shown in Figure 3. It can be seen that the rate of protein synthesis by the lung increased as the pH was increased from 6.6 to 7.6 where the maximum rate of protein synthesis was observed. Increasing the pH from 7.6 to 8.2 resulted in a decrease in the rate of protein synthesis.

5- The Specific Effect of pH on CSF Production: A more realistic view on the pH dependence of CSF production by the lung was obtained by examining the changes in specific production of the CSF as a function of the pH. The specific production was defined as the ratio of number of colonies produced at a given pH to the cpm of the related CSF sample at

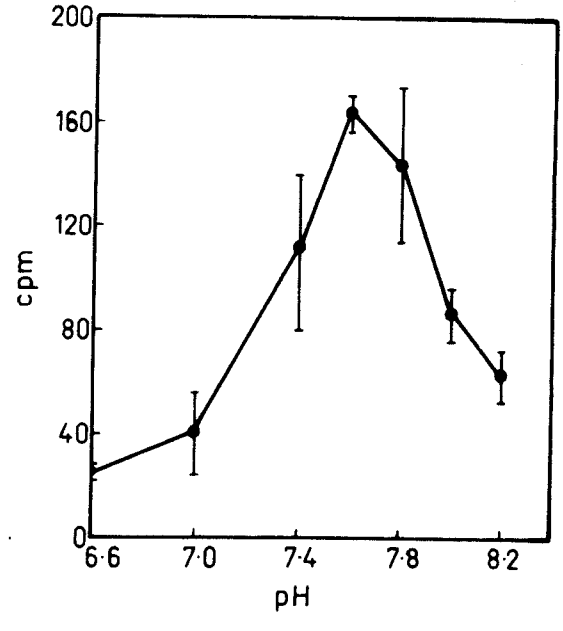

Figure 3- The effect of environmental pH on the synthesis of releasable proteins by the lung. Mean ± SE of two experiments.

the same pH. The specific production of CSF as a function of pH is shown in Figure 4. The data from Figures 1 and 3 were used in an arbitrary scale to generate Figure 4. It can be seen from Figure 4 that the maximum specific production of CSF was observed at pH 8.0.

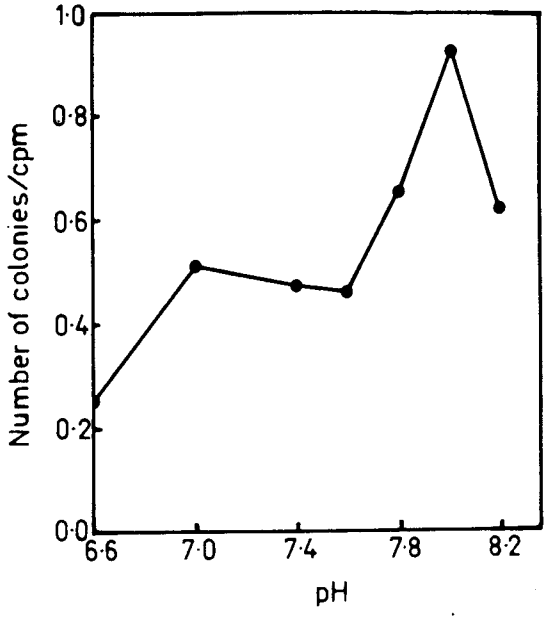

Figure 4- The specific production of CSF by the lung as a function of pH. The data from Figures 1 and 3 are used to generate this figure.

Discussion

We have studied the effect of environmental pH on the production of CSF by the lung. The culture medium used in this study, DMEM, was buffered by Na^+ -bicarbonate system. In this system two alternative approaches could be used to change the pH; 1- keep the bicarbonate concentration constant and change theCO_2 partial pressure in the atmosphere of the incubator, or 2- keep the CO_2 constant and change the bicarbonate concentration in the medium. We used the second alternative because the results were highly reproducible. However, the bicarbonate system had a limited pH range. On the acidic side, the minimum obtainable pH was 6.6. While, on the basic side the maximum pH which this system could tolerate was 8.2. Therefore, the range of pH studied here was 6.6 to 8.2 In this range the CSF production depended on the pH of the culture medium.

Adjusting the pH by varying the NaHCO3 concentration in the medium resulted in different salt concentrations in each sample. Therefore, it was necessary to rule out the possibility of a Na^+ concentration effect in these experiments. The results of Figure 2 clearly excluded this possibility and showed that the variations observed in CSF production was due to the variations in the pH of the culture medium and not to the Na^+ concentration.

CSFs are glycoproteins synthesized by the lung and other tissues in the culture medium. Therefore, it was interesting to find out if the observed effect of pH on the CSF production was merely a result of the effect of pH on the protein synthesis by the lung. Since CSFs are synthesized and subsequently released in the medium, we measured the effect of pH on the rate of synthesis of releasable proteins by the lung. This was done by measuring the radioactivity incorporated into the conditioned medium of the lung. The results shown in Figures 3 and 4 suggest that *in-vitro* protein synthesis by the lung was affected by the pH of the medium. However, a specific effect of pH on the CSF production could be demonstrated by calculating and plotting the specific production of CSF.

The specific production is a suitable parameter to evaluate the specific effects of pH on CSF production. If an agent stimulates or inhibits protein synthesis non-specifically, it is expected that all various proteins be stimulated or inhibited to the same extent. Therefore, a nearly constant value of the specific production could result at various concentrations of that agent. In our case, the specific production curve as a function of pH was not linear. There was a maximum at pH 8.0 which indicated that there was a specific stimulation of CSF at this pH with respect to other proteins.

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