The Turkish Journal of Pediatrics
1973 / Vol. 15 / No. 4 / pp. 179 - 185

With Reference to the Failure of the Lymphocyte Blastic Transformation in Chronic Lymphocytic Leukaemia (CLL): A Study on the Effect of Treatments*

G. Astaldi / B. Yalçın*** / P. L. Bargero / P. Paleani Vettori**** / E. Bologna***** / R. Merolla, / P. Comi*****

Introduction

For several years it has been shown that peripheral-blood lymphocytes from CLL undergo quite a low extent of blastic transformation in a 3-day cell-culture stimulated either with PHA[3,17] or other plant mitogens (PKM or CON-A) as well as with other agents.[6] The reason for this failure is not yet fully clarified, though functional and ultrastructural studies of CLL-lymphocytes incubated *in vitro* with different mitogens up to 11 days suggest that the above-mentioned lymphocyte population is comprised predominantly of abnormal cells in addition to a small number of normal T and B lymphocytes.[8, 9, 10, 11, 15]

---

* Supported by "The Blood Research Foundation", Washington D. C. / USA
Paper delivered at the Immunology Symposium: *Advances in Pediatric Immunology*, held in Ankara/Turkey, Sept. 1, 1973, at the IX Mediterranean and Middle-Eastern Pediatric Congress.

** Director, The Blood Research Foundation Center, Tortona Italy.
*** From the Department of Internal Medicine, the Cerrahpasa Medical Faculty, Istanbul, Turkey (Director: Prof. O. Barlas).
**** From "Consiglio Nazionale Ricerche (C. N. R.), Laboratorio Tecnologie Biomodiche", Roma (Director: Prof. P. Paleani Vettori).
***** From the Clinical Pathology Department, Hospital of San Remo, Italy (Head: Dr. A. Siccardi).

It also appears that the small portion of normal lymphocytes is endowed with normal blastic behavior, including mitotic activity[2], and the capability to participate into the skin delayed-allergy reaction[4, 5]

After the development of the immune fluorescence technique[20], lymphocytes from patients with CLL have been recently examined also for the presence of easily detectable surface-bound immunoglobulins and for their ability to form spontaneous "non-immune" rosettes with sheep red blood cells (SRBC), most probably a marker for T-cells[14]. So far, these studies showed marked differences among the individual patients. In fact, whereas the percentages of circulating B-cell-like lymphocytes in the majority of patients with CLL is significantly increased[9, 13, 18, 19, 23], and the proportion of lymphocytes forming spontaneous rosettes with SRBC is reduced as compared with normal subects[8] in other CLL-patients the results were just the opposite. In other words, the ratio between the surface Ig-carrying lymphocytes (B-cells) and the surface Ig-lacking ones (T-cells) in the peripheral blood from CLL may differ so markedly from patient to patient that two distinct groups of CLL-patients may be detected: one with decreased, and one with increased numbers of lymphocyte carrying Ig on their surface. Nevertheless no correlation could be proven until now, between the surface Ig-pattern of peripheral blood lymphocytes from CLL and their blastogenesis extent in a 3-day cell-culture stimulated with plant mitogens[21].

In order to further investigate the defective behaviour of CLL-lymphocytes in front of antigen stimulation, we have now studied the effect of treatment - either chemotherapy or ionizing irradiation of the spleen - on the blastic transformation of those lymphocytes. Preliminary observations have shown indeed that spleen ionizing irradiation may influence the blast percentages which occur in the PHA-cell cultures of CLL-lymphocytes.[1]

Material and Methods

Peripheral-blood lymphocytes obtained from patients with CLL were set up in the 3-day cell-culture system and stimulated with PHA. In a few patients, also PKM was used as the stimulating agent. After 3 days of incubation at 37°C, smears of the cultures were made and stained with May-Grünwald-Giemsa. Then, 1000 cells per culture were examined and the percentages of the transformed lymphocytes determined. This observation was performed either before, or several times during and after the patient's treatment.

A first group of patients was submitted to chemotherapy with the following antiblastic drugs: Chlorambucil, Peptichemio, Trenimon,

Corticosteroid Hormones. A second group of patients was submitted to spleen ionizing irradiation which was carried out with x-ray in all patients but one. This patient was treated with $Co.^{60}$

Results

Before treatment, the rate of lymphocyte blastogenesis was quite low in all the examined CLL-patients, either when the stumilation was done with PHA, or with PKM. The average values were in fact 4.3 ± 3.6 and 5.0 ± 4.1, respectively.

During and after the tested chemotherapy, no significant increase of blast percentages were observed in our lymphocyte cultures, both PHA-and PKM-ones. In contrast, the blastogenesis extents appeared significantly higher after spleen irradiation in all patients except one, sometimes reaching rates which are usually obtained with peripheral-blood lymphocytes from normal donors. By grouping the irradiated patients according to their scheme treatment the following was observed:

- Three patients received a single dose of 300 r. One of them failed to respond, whereas two reached the maximal percentage of 30 blast-like cells around the 10th day after spleen irradiation. The return to the original values occured approximately about the 20th day.
- Three patients received 2 weekly doses of 100 r each, until their total doses ranged from 500 to 600 r. The culture-blastogenesis improvement was observed in all these subjects with maximal blast percentages ranging from 40 to 50 between the 21st and the 40th day after the spleen irradiation onset. The original rates reappeared after 1-3 months.
- Six patients received 3 to 5 weekly doses of 100 r each with a total of 400 to 500 r. The improvement of lymphocyte-blastogenesis occured in all these 6 patients with maximal blast percentages ranging between 42.5 and 80. Those peaks were observed between the 10th and the 30th day after the irradiation onset, whereas the return to the original rates was observed after 1-2 months.

The cultures of 2 of these patients were stimulated either with PHA or with PKM. It was possible to observe that: (a) before irradiation, both the tested stimulants gave similar reduced blastogenesis extents, in comparison to those extents obtained with lymphocytes from normal donors (b) after spleen irradiation, the blastogenesis rates

increased approximately in the same manner for both the above-mentioned mitogens.

– One patient was finally irradiated with $Co,^{60}$ and he did receive 5 weekly doses of 100 rads each, for a total of 1,700. The maximal peak of blast-like cells was 92%, and was observed on the 40th day after the onset of spleen-irradiation. Normal blastogenesis extents, such as 70-80% continued for about 4 months after the beginning of treatment. Then, these values started to decrease, but rates around the original ones appeared just one year after the $Co.^{60}$ irradiation.

Discussion and Conclusions

The results of this study do not show any marked effect of chemotherapy on the defective responsiveness of the peripheral-blood lymphocytes from CLL, when they are stimulated with plant mitogens in the 3-day cell-culture system. This refers at least to the drugs tested by us, namely: Chlorambucil, Peptichemio, Trenimon, and Corticosteroids. In contrast, spleen ionizing irradiation significantly increased the lymphocyte-culture blastogenesis in all but one of the 13 CLL-patients thus treated. This intense lymphocyte blastogenesis lasted from two weeks up to several months in the x-ray irradiated patients (300-600), and one year in the patients irradiated with 1,700 rads of $Co.^{60}$

From the results, taken as a whole, apparently a relationship does exist between dose and scheme of irradiation, and the entity and duration of the increased responsiveness of lymphocytes to antigen stimulation. Besides, a relationship should also exist between the extent of lymphocyte response on one hand, and the spleen size and the lymphocyte count and individual variability, on the other. Also, the lymphocyte immunological pattern of the single cases should interfere with entity and duration of the irradiation effect and in particular, the ratio between surface Ig-carrying and Ig-lacking lymphocytes as well as the frequency of the predominant immunologically *abnormal* (leukemic) cells, which are intermingled with the less numerous *normal* T- and B-cells in the peripheral blood of CLL.

By comparing the results of the PHA- and PKM-cultures, it appears that the lymphocytes of our CLL-patients did behave in the same manner in regard to both of the tested plant mitogens, either before, or during and after spleen irradiation. As it was reported, the CLL-lymphocytes undergo active blastogenesis after PKM-stimula-

tion,[23] and this leads one to believe that the above-mentioned lymphocytes were immunologically normal B-cells. Our results, however, do not agree with these data and interpretation. Rather, they are in agreement with other recent experiments[16, 22] showing that CLL-lymphocytes fail to respond not only to PHA, but also to PKM and that they are immunologically *defective* or *abnormal* lymphocytes rather than *normal* B-cells.

Finally, we would like to briefly discuss the possible mechanism(s) by which splenic ionizing irradiation brings back toward a normal rate the defective blastogenesis of the CLL-lymphocytes stimulated by plant mitogens in the 3-day cell-culture. A possible answer could be that the abnormal lymphocyte clone of CLL is prevalently localized in the spleen; it would, therefore, come up against a marked - even if temporary - reduction in quantity owing to the irradiation effect on that organ. In that case, the ratio between the *abnormal* (leukemic) lymphocytes and the *normal* ones in peripheral-blood would be shifted in favor of the latter. Another hypothesis is that ionizing irradiation modifies the cellular membrane of the immunologically abnormal leukemic lymphocytes either with regard to their electric charge or, rather, with regard to the surface-bound immunoglobulins which are specifically assigned to promote responsiveness to mitogens. A further possible mechanism could be connected with the fact, that ionizing irradiation "in vivo" (and not "in vitro") may markedly stimulate the release of the lymphocyte-activating factor,[12] which either potentiates the mitotic response of thymus lymphocytes to PHA, or significantly increases the incorporation of 3H-Tdr by thymus lymphocytes in the absence of PHA. Only further studies will be able to supply the proper answer to the interpretative assumptions advanced here, or to others that could be put forward in the future.

Acknowledgments

The technical assistance of Dr. Marco Tasca and Dr. Gustavo Ottolenghi in the patients' irradiation, as well as that of Miss Gianna Campora in setting up the cultures is gratefully acknowledged.

REFERENCES

1. Astaldi, G., Airò, R.: Phytohaemagglutinin and human lymphocytes in short-term cell culture. In: Bristol Symposium on the Lymphocyte in Immunology and Haemopoiesis. Ed. J. M. Yoffey. Arnold Ltd., London 1966, pag. 73.
2. Astaldi, G., Airò, R.: With reference to the PHA-hypo-responsiveness of lymphocytes from chronic lymphocytic leukemia in cell culture. Souvenir of IV Asian-Pasific Congress of Hematology. Ass. Adv. Printers, Bombay, 1967, pag. 57.

3. Astaldi, G., Airò, R., Sauli, S.: In vitro studies on leukaemic cells. In: Current research in leukaemia. Ed. F. G. J. Hayhoe. The Syndics of the Cambridge Univ. Press, London 1965, pag. 139.

4. Astaldi, G., Burgio G. R., Genova, R., Curtoni, E.: Histo-Cytology of phytohaemagglutinin skin reaction in healthy persons and in patients with chronic lymphocytic leukaemia. Atti del 7° Congr. Int. Accademia Int. di Patologia Accademia Nazionale dei Lincei, Roma 135: 583, 1970.

5. Astaldi, G., Burgio, G. R., Genova, R., Curtoni, E., Giraudo, L. C.: Histological skin reaction to intradermal inoculations of pokeweed to humans. J. Reticuloendothelial Soc. 7: 126, 1970.

6. Astaldi, G., Lisiewicz, J.: Lymphocytes: Structure Production, Functions. Idelson, Naples,1971

7. Cohnen, G., Augener, W., König, E., Brittinger, G., Douglas, S. D.: B lymphocytes in Hodgkin's disease. New England J. Med. 288: 161, 1973.

8. Cohnen, G., Douglas, S. D., König, E., Brittinger, G.: Pokeweed mitogen response of lymphocytes in chronic lymphocytic leukemia. A fine structural study. Blood (in press).

9. Cohnen, G., König, E., Augener, W., Brittinger, G., Douglas, S. D.: Lymphocyte populations in chronic lymphocytic leukemia. Lancet i: 259, 1973.

10. Douglas, S. D., Cohnen, G., Brittinger, G.: Ultrastructural comparison between phytomitogen transformed normal and chronic lymphocytic leukemia lymphocytes. J. Ultrastruct. Res. (in press).

11. Douglas, S. D., Cohnen, G., König, A., Brittinger, G.: Ultrastructural features of phytohemagglutinin and concanavalin A - responsive lymphocytes in chronic lymphocytic leukemia. Acta Haemat. (in press).

12. Geiger, B., Gallily, R., Gery, I.: The effect of irradiation on the release of lymphocyte - activating factor (LAF). Cellular Immunology 7: 177, 1973.

13. Johansson, B., Klein, E.: Cell surface localized IgM - kappa immunoglobulin reactivity in a case of chronic lymphocytic leukemia. Clin. Exp. Immunol. 6: 421, 1970.

14. Jondal, M., Holm, G., Wigzell, H.: Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming non immune rosettes with sheep red blood cells. J. Exp. Med. 136: 207, 1972.

15. König E., Cohnen, G., Brittinger, G., Douglas, S. D.: Response to phytohaemagglutinin and pokeweed mitogen in chronic lymphocytic leukaemia. Lancet i: 795, 1972.

16. Moore G. E., Minowada, J.: T or B lymphocytes in chronic lymphocytic leukemia. Lancet i: 38, 1972.

17. Nowell, P. C.: Differentiation of human leukemic leukocytes in tissue culture. Exp. Cell Res. 19: 267, 1960.

18. Papamichail, M., Brown, J. C., Holborow, E. J.: Immunoglobulins on the surface of human lymphocytes. Lancet ii: 850, 1971.

19. Pernis, B., Ferranini, M., Forni, L., Amante, L.: Immunoglobulins on lymphocyte membranes. In: Progress in Immunology. Ed. B. Amos. Acad. Press, New York 1971, pag. 95.

20. Pernis, B., Forni, L., Amante, L.: Immunoglobulin spots on the surface of rabbit lymphocytes. Exp. Med. 132: 1001, 1970.
21. Piessens, W. F., Schur, P. M., Moloney, W. C., Churchill, W. H.: Lymphocyte surface immuno-globulins. Distribution and frequency in lymphoproloferative diseases. The New England J. Med. 288: 176, 1973.
22. Smith, J. L., Cowling, D. C., Burker, C. R.: Response of lymphocytes in chronic lymphocytic leukaemia to plant mitogen. Lancet i: 229, 1972.
23. Wilson, J. D., Nossal, G. J. V.: Identification of human T and B lymphocytes in normal peripheral blood and in chronic lymphocytic leukeamia. Lancet ii: 788, 1971.