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Behaviour of Lymphocytes from Cancer Patients and Normal Individuals Cultured with Phytohaemagglutinin *

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Blood lymphocytes from cancer patients with solid tumours without any previous immunosuppressive treatment and from normal individuals, were cultured *in vitro* with a wide range of phytohaemagglutinin (PHA).

Sixty two per cent of all the cancer patients studied show a minimal of no response to PHA in comparison with the normal population. The rest (38 %), show a quantitative identical response than normals. However, the maximal response in these patients occur in the high PHA doses, while the normal individuals show their maximal activity with low PHA doses.

The low or no PHA response showed by the 62 % of patients, may indicate they have impaired cellular immunity. The high response showed by the other 38 %, may indicate that the patients of this group have high cellular immunity capacity. This immunity, however, higher PHA doses are required to reach the maximal response compared with the seems to be diferent from that of normal individuals, since higher PHA doses are required in cancer patients to reach maximal response. These results also suggest that a large range of PHA doses may be important to detect the degree of cellular immunity in cancer patients compared with the normal population. One or two randon PHA doses, may not show a distintion.

It has been proposed that the immune system plays an essential role in surveying for mutant neoplastic cells (2, 3). When peripheral lymphocytes are cultured *in vitro* with phytohaemagglutinin (PHA) they are transformed into blast cells which undergo DNA replication and mitosis (7, 9, 11). As it has been shown that there is a close relationship between the intensity of the lymphocytes responsiveness against PHA and the funtional capacity of the immune system, this model has been used for many authors to measure the competence of the immune system in cancer patients.

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Opposite results have been obtained when the behaviour of lymphocytes from patients with solid tumours, cultured with PHA, has been compared with the behaviour of lymphocytes from normal individuals (5, 7, 8, 12, 13).

The present paper shows the results of an attempt to study again this problem in patients with solid tumours and normal individuals.

Materials and Methods

Peripheral blood lymphocytes from 22 cancer patients with solid tumours selected without any previous immunosupressive treatment and from 14 normal individuals, were cultured with six diferents doses of pytohaemagglutinin (PHA). The average ages of each group were similar.

The lymphocytes were obtained by the BÖYUM technique (1) using «Lymphoprep» (Nyegard, Norway) from 20 mil of heparinized blood sample. The cells were washed twice in Eagle's medium (Wellcome and the cell concentration was adjusted to 1×10^6 lymphocytes/ml in Eagle's medium plus 10 % foetal calf serum heated at 56° C for one half hour and 100 μg/ml of sodium cloxacillin and 100 μg/ml of ampicillin. The suspension was dispensed in aliquots of 1 ml into the culture tubes. Serial dilutions of PHA Regent grade, Wellcome) were done and 20 μl were added per culture tube to make the final concentration of 0.006, 0.0125, 0.025, 0.05, 0.1 and 0.2 mg/ml on the assumption that one PHA vial contain 40 mg of PHA activity. The cultures were incubated for 3 days at 37° C in an atmosphere rich in CO_2 (94 % air plus 4 % CO_2) as described by FESTENSTEIN (6). At the end of 3 days, 20 μl of thymidine-C^{14} (0.16 μCi) was added to each culture tube for 16 hours. The cultures were harvested and the radioactivity uptake was measured according to the technique of FESTENSTEIN (6) in a liquid scintillation counter (Unilux, Nuclear Chicago).

Results

The results (fig. 1) show two distint groups of cancer patients according to the grade of response to phytohaemagglu-

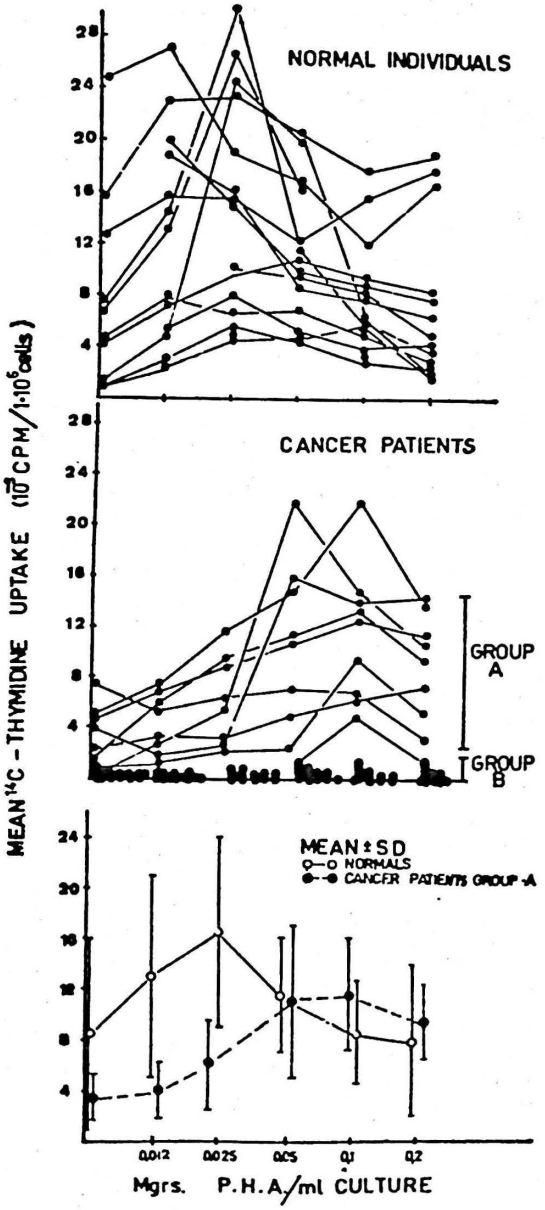

Fig. 1. ^{14}C-thymidine incorporation of peripheral lymphocytes from normal individuals, cancer patients and the mean ± S.D. of normals and group A of cancer patients.

tinin. They are groups A and B. The group A, which comprises 38 % of the total cancer patients studied, has a good response in comparison with the normal population. The group B, comprises 62 % of the total and has minimal or no response.

The pattern of response showed by group A is not identical to the normal individuals. These cancer patients need higher PHA concentration to reach the maximal response than the normals, who show their maximal response with lower PHA concentrations.

Discussion

There seems to be no agreement on how lymphocytes from patients with solid tumours behave when they are stimulated *in vitro* with PHA, as compared with lymphocytes from normal individuals. Some authors have reported lower response to PHA in lymphocytes from cancer patients (5, 7, 8) while others did not find any significant difference (12, 13) between lymphocytes from normal and cancer patients.

As the above experiments were carried out using one or two PHA doses, the present work was done using a wide range of PHA doses. In each case a pattern of PHA response was obtained, thus this method seems to give a more accurate representation of *in vitro* lymphocytes activity with PHA than when one or two standard doses were used.

Two degrees of PHA response in cancer patients with solid tumours has been reported in comparison with the normal population. The lower response, group B, may indicate that these patients have impaired immunity. As the lymphoid cells which respond to PHA are thymus dependent (4) and they are responsable for the cell-mediated reactions (10), the immunological impairement of the patients seems at least to affect the cell-mediated immunity.

The higher PHA response in group A could mean they have normal immunological function, at least in cell-mediated immunity. However these patients show a different pattern of response than the normal individuals, they demostrate maximal activity in the higher PHA concentrations in contrast to the normal population which shows maximal activity in the lower PHA concentrations. These results suggest that the contradictory findings which have been published (5, 7, 8, 12, 13) may be explained on the bassis of the number of lymphocytes and the amount of PHA which has been used. For example, with PHA doses of 0.0125 and 0.025 mg/ml (fig. 1) the response would be higher in the controls, whereas the response would be equal in control and cancer patients in the 0.05, 0.1 and 0.2 mg/ml PHA doses. These findings could be explained on the basis that T-cells population in these cancer patients is less sensitive to PHA than in normal individuals, but further studies will be necesary in orden to confirm this hypothesis.

From the practical point of view, the results obtained in both groups of cancer patients studied in this comunication suggest that a large range of selected PHA concentrations may be important to detect the degree of cellular immunity in cancer patients compared to the normal population. Given the importance to cell mediated mechanisms in the suppression of malignant cells (2, 3), this seems to be a positive advance in the diagnosis of cancer and also in the pronosis of these patients.

Resumen

Se han cultivado *in vitro* linfocitos de sangre periférica, obtenida de sujetos normales y de pacientes con tumores sólidos sin tratamiento inmunosupresivo previo, frente a una amplia escala de dosis de fitohemaglutinina (PHA).

El 62 % del total de enfermos estudiados mostraron mínima o ninguna respuesta frente a la PHA en comparación con la población

normal. El resto (38 %) mostraron una respuesta cuantitativamente idéntica a los normales. Ahora bien, la máxima respuesta en estos pacientes se presentó empleando dosis elevadas de PHA, mientras que la máxima respuesta en sujetos normales ocurre en las dosis más bajas.

El bajo o nulo grado de respuesta frente a la PHA presentado en el 62 % de pacientes podría indicar una falta funcional de su sistema inmunológico de tipo celular. La alta respuesta presentada por el 38 % restante indicaría que los enfermos pertenecientes a este grupo presentan un sistema inmune de tipo celular con un alto nivel funcional. Sin embargo, el sistema inmune de estos pacientes parece ser diferente del normal, dado que son necesarias mayores concentraciones de PHA para obtener su máxima respuesta. Estos resultados también sugieren que es importante emplear una amplia escala de concentraciones de PHA para detectar el grado funcional del sistema inmune de tipo celular en enfermos cancerosos.

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