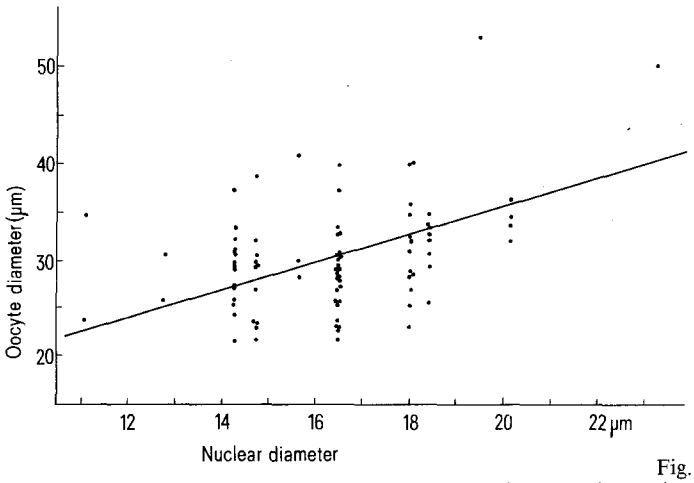

Fig. 2. The relation of the size of the oocyte to that of the respective nucleus in the foetal human ovary. The line shown is calculated from the regression: $y = 1.46\,x + 6.77$, $r = +0.51$; $p < 0.001$.

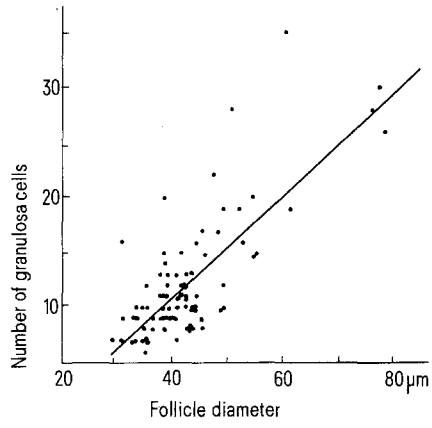

Fig. 3. The number of granulosa cells in the widest cross-section of the follicle and follicle diameter in the foetal human ovary were positively and linearly correlated: $y = 0.46\,x - 7.39$; $r = +0.51$; $p < 0.001$.

follicle size. Howewer, in all ovaries examined, regardless of age of foetus (from 5 month p.c. onwards), small antral follicles with mean diameters ranged from 0.20 to 0.40 mm and with about 100–160 granulosa cells were detected.

The occurrence of antral follicle has been reported by other authors[2-9] for newborn and foetal ovaries from 7 months p.c. onwards. In our experience, this striking characteristic of foetal human ovary is confirmed and extended until to 21 weeks of foetal life. The presence of antral follicle did not seem to be related to any patological status of parents. Particularly, none of the mothers of foetuses examined were by affected by diabetes. Therefore, we did not confirm the hypothesis of Alvin and Bauer[6] which suggested a relationship between the presence of antral follicles in the newborn ovary and diabetes in the mother. Probably, endocrine factors arising from maternal blood or from placenta are mainly responsible for antral follicle growth in the foetal human ovary.

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Inhibition or augmentation of PHA-induced lymphocyte transformation by factors of cultured lymphoblastoid cell lines[1,2]

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Summary. Stimulation of human peripheral blood lymphocytes by phytohemagglutinin (PHA) was found to be suppressed or augmented by the addition of supernatants or cell dialysates of cultured lymphoblastoid cell lines.

Human peripheral blood lymphocytes in vitro produce a variety of soluble factors upon stimulation by mitogens or antigens[3]. Established lymphoblastoid cell lines morphologically resemble these stimulated lymphocytes[4], have surface properties characteristic of bone marrow-derived (B-cells) or thymus-derived (T-cells) lymphocytes[5], and produce a variety of products[3].

In the present work, findings on soluble factor(s) derived from T- or B-lymphoblastoid cell lines capable of augmenting or suppressing the capability of human peripheral blood lymphocytes for mitogenic reactivity by PHA are reported.

Materials and methods. The following human B-cell lines were used: 1. IIBR-3: established in our laboratory, 2. Namalva[6], 3. LDV/7[7]. The T-cell lines: Molt-3 and Molt-4F[8]. The characterization of the B- and T-cell lines was done as described by Minowada et al.[8]. Cells of all lymphoblastoid cell lines were grown in agitated suspended culture at 36.5 °C.

Supernatants from lymphoblastoid cell culture (2×10^6 cells/ml) were collected. Simultaneously the cells (10^7 cells/ml) were disrupted by rapid freezing and thawing process and the dialyzable fractions collected[9]. The mixed lymphoblastoid cell line cultures were done by equal volumes of Molt-4F and Namalva, each containing 5×10^5 cells/ml. Molt-4F mixed culture with sheep red blood cells (SRBC) was prepared by equal volumes of Molt-4F (2.5×10^6 cells/ml) and SRBC (10^7 cells/ml). The cultures were propagated for 5 days. All samples were stored at −20 °C until tested for activity.

Human peripheral blood lymphocytes from 4 healthy donors were used for blastogenic transformation. The

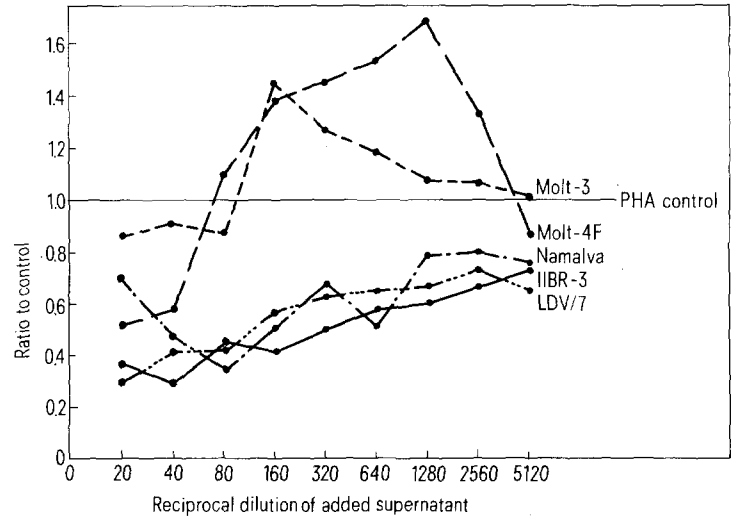

Fig. 1. Mitogenic response by PHA of human lymphocytes treated with supernatants of 5 lymphoblastoid cell lines at different concentration. Each point represents the average incorporation ratio of ^{3}H-thymidine of triplicate. The initial concentration prepared from 2×10^6 viable cells/ml.

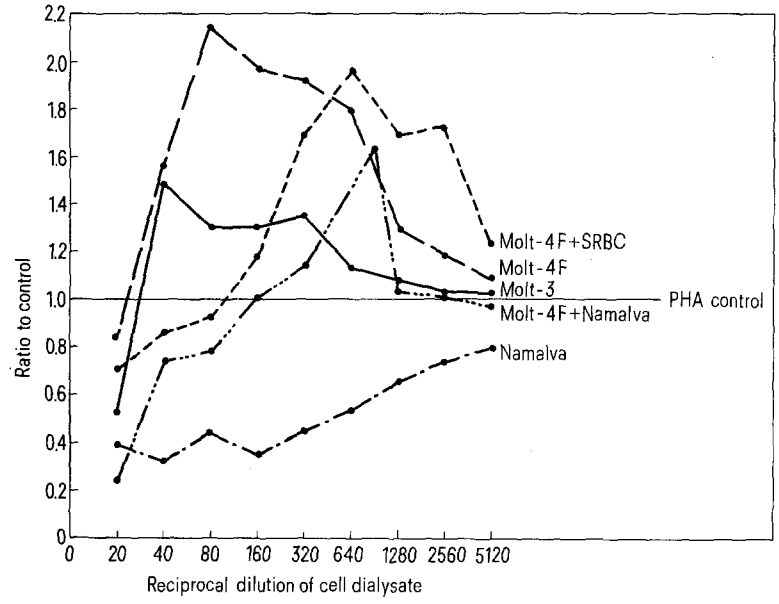

Fig. 2. Mitogenic response by PHA of human lymphocytes treated with freeze-thawed lymphoblastoid cell dialysates at different cultivation methods. Each point represents the average incorporation ratio of ^{3}H-thymidine of triplicate. The initial concentration of dialysates prepared from 10^7 viable cells/ml.

defibrinated blood was layered on a Ficoll-Hypaque gradient[10] and the leucocyte layer collected. For each donor the base mitogenic concentration line of PHA stimulation was determined.

Samples of supernatants or cell dialysates were diluted in RPMI 1640 medium + 10% FCS and added to cultures of human peripheral blood lymphocytes in 2 ml volume (2×10^6 cells/ml). The cells were incubated (37 °C, 5% CO_2) for 24 h, spinned down and supernatant discarded. New cell cultures were set up containing 2.5×10^5 viable cells/ml with PHA and in parallel without PHA. The PHA concentration was 70% of the maximal mitogenic response of the cells as tested for each donor. Cultures were incubated for additional 72 h. 1 µCi ^{3}H-thymidine was added in each tube 16 h before cell harvesting[11]. Transformation ratio was calculated as the relationship between incorporation of ^{3}H-thymidine in PHA transformed cultures treated with supernatants or cell dialysates and the background of the same treatment without PHA.

Results and discussion. Preincubation of human peripheral lymphocytes with supernatants of cell culture of T-lymphoblastoid cell lines (Molt-3 or Molt-4F) caused a markedly increased reactivity in the presence of PHA when compared to cells preincubated only in control medium. Moreover, the increased mitogenic reactivity was limited to supernatants from these T-cell lines and not to supernatants of the investigated B cell lines (figure 1). All dialysates from Molt-3, Molt-4F, Molt-4F cultured with SRBC and Molt-4F cultured with Namalva have the same augmenting properties. Cell dialysates from Namalva alone (B-cell line) suppressed the PHA-induced transformation in all tested concentrations (figure 2).

Lymphocyte mitogenic response induced by PHA has provided a widely used technique for estimating immunocompetence of the cellular immune system[12]. The observation reported here establishes that the mitogenic response of normal human lymphocytes to a nonspecific mitogen (PHA) is enhanced by preincubation of these lymphocytes in supernatant or cell dialysates from 2 human T-lymphoblastoid cell lines (Molt-3 and Molt-4F). Under the same experimental conditions supernatants or cell dialysates of 3 tested B-lymphoblastoid cell lines (IIBR-3, Namalva, LDV/7) suppressed the mitogenic reactivity of normal lymphocytes.

It was reported that mature T-cells have a relatively higher reactivity to PHA[13] and thymus humoral factor increases the mitogenic reactivity of normal spleen cells [1] There is an

ever-growing list of inhibitors of lymphocyte blastogenesis produced by various lymphoid cells or extracts[14-17].

In our experiments the effector substance from the supernatants was not dialysable. Only the dialysates of freeze-thawed cells had the same enhancing or depressing effect. At the present stage of the research, it is not clear whether one or more substances are involved. Further physicochemical and biological investigations are critically important. Such a study might improve our understanding of the regulatory control mechanisms which are operative in humoral and cellular immunity.

1 This research was supported by a grant from the United States-Israel Binational Science Foundation (BSF), Jerusalem, Israel.
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Different marrow cell number requirements for the haemopoietic colony formation and the cure of the W/W^v anemia

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Summary. The lowest cell number in the normal marrow transplant, which allows the cure of W/W^v anemia was found to be between 10^4 and 10^5. This exceeds by several times the lowest cell number necessary for the haemopoietic colony formation. Therefore, either the colony forming cell is not the haemopoietic stem cell but rather its progeny, or this cell requires an aid from some other cells to exert its activity.

The haemopoietic stem cells (HSCs) are functionally defined by 2 properties secondary to their capacities for self-renewal, differentiation and extensive proliferation[2]. One of these properties is a spleen colony formation[3], the other is the establishment of continuous haemopoiesis. These properties may be assayed by transplantation of cell suspensions (e.g. marrow) containing HSCs into HSC-deprived mice, like lethally-irradiated mouse of any genotype or the W/W^v anemic mouse with genetically-inherited stem cell deficiency[4]. In this latter system, marrow from conisogenic +/+ haematologically normal littermates forms spleen colonies in unirradiated W/W^v recipients[5], and the establishment of continuous haemopoiesis by grafted cells may be observed as the cure of anemia[6]. Although the establishment of continuous haemopoiesis is believed to be a direct consequence of the spleen colony formation, no experiment was reported to date on the relationship of cell number requirements for these 2 HSC assays.

Due to poor survival, the lethally-irradiated mouse is a very inconvenient model for studies with limiting dilutions of stem cells, and the use of W/W^v mouse overcomes this difficulty as it is near to normally viable[7]. Theoretically, a single HSC should cure the W/W^v anemia, and, basing on the colony forming unit in the spleen (CFU-S) assay corrected for seeding efficiency in both spleen[8] and marrow[9], less than 2000 marrow cells should supply one implanted HSC. On the other hand, we have recently described a cellular element operationally termed the 'anti-theta sensitive regulatory cell' (TSRC), which is necessary to synergize with the HSC in curing the W/W^v anemia, although it has no effect on the number of spleen colonies formed by the same marrow cells in W/W^v recipients[10]. We therefore theoretized that the assay for the cure and the CFU-S assay may not lead to the similar quantitative estimation of the HSC.

Materials and methods. WBB6F$_1$ mice of both W/W^v and +/+ genotype were bred in the Animal Facility, Postgraduate Center, Military School of Medicine, Warsaw, Poland, by mating WB/Re-W/+ and C57B1/6-W^v/+ parents. Groups of 5–10 W/W^v male mice (2–3 months of age) were injected i.v. with numbers of +/+ marrow cells ranging from 10^2 to 10^7. Every 3 weeks post-transplant for

The spleen colony formation and the cure of the W/W^v anemia following the transplant of various doses of conisogenic +/+ normal marrow cells

Dose of transplanted marrow cells	Number of 8-day spleen colonies formed by these cells ($\bar{x} \pm SE$)	Number of cured** animals/number of transplanted animals
10^7	-*	5/5
10^6	-	5/5
10^5	21.4 ± 3.1	5/5
10^4	2.0 ± 0.3	4/10
10^3	-	1/10
10^2	-	0/10

* Not studied. ** The cure, if happened persisted through the whole observation period of 6 months.