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.

6 months, the recipient mice were bled from the retroorbital sinus for the determination of peripheral blood values. The cure of their anemia was individually diagnozed, if they acquired haematological values typical of +/+ marrow donors using the previously described criteria[11]. These criteria utilize the red cell size as the genetic marker for the origin of red cells present in the recipient's circulation. The separate groups of 5 W/W^v recipients received either 10^4 or 10^5 marrow cells, and the number of spleen colonies formed by this marrow was determined 8 days post-transplant using the method of Till and McCulloch[3].

Results and discussion. As is shown in the table, all the recipients of 10^5, 10^6 and 10^7 +/+ marrow cells became cured of their anemia. The observation that only 4 out of 10 recipients of 10^4 +/+ marrow cells became cured, suggests that the lowest number of cells required for the cure is in the range of 10^4 to 10^5 and most probably equals approximately 2.5×10^4 cells. On the other hand, 10^4 +/+ marrow cells formed 2.0 ± 0.3 colonies in the spleen. From studies of Wolf and Trentin[12], it is known that, except for the spleen, the haemopoietic colonies are formed in the marrow and that number of marrow colonies doubles in the spleen. Altogether, these data suggest about 10-fold difference between the marrow cell number required for the cure of the W/W^v anemia and the haemopoietic colony formation. This may suggest that the HSC as defined by the CFU-S assay is not the only prerequisite of the cure of the W/W^v anemia. As according to Abramson et al.[13] it is possible to populate W/W^v haemopoietic tissues with the progeny of a single stem cell, the present data suggest that either the CFU-S is not this cell or it requires for its functional activity the cooperation of some other factor present in the grafted material. This goes well with the concept of regulatory influence of the TSRC. It was previously shown that the TSRC regulates the CFU-S self-renewal[11,14] and partially its differentiation[11].

Another alternative is Schofield's hypothesis[15] that the true HSC is a cell, fixed in appropriate cellular environment called a stem cell 'niche' and retaining its capacity for indefinite self-renewal. If it leaves the niche or fails to lodge into one following the transplant, it becomes the CFU-S cell. The CFU-S cell is subsequently defined as a pluripotent cell with limited reproductive ability. However, if the CFU-S finds the niche it may become the HSC. As according to this concept the CFU-S is a stem cell daughter, it should be more common in the marrow than the functional stem cell. This would also explain why less marrow cells are required for colony formation than for the cure of the W/W^v anemia. However, the consequence of this concept is that the number of CFU-S cells which would find the niche and subsequently exert the HSC capacities depends on the quantitative relationship between the number of transplanted stem cells and the number of niches available. Since in our system, there was an enormous excess of niches above the number of transplanted stem cells, this concept requires that under such conditions most of the transplanted CFU-S cells would become stem cells. Therefore, the difference between cell number requirements for the cure and for the colony formation would be expected to be minimized. This was not the case in our experiment. Further studies are necessary to resolve these disparities.

1 Acknowledgments. We are indebted to Doc. Dr Maksymilian Siekierzynski for his help and advice and to Miss Elzbieta Szewczyk for expert technical assistance.

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A negative association of HLA-BW52 with Graves' disease and insulin-dependent diabetes mellitus with juvenile onset among Japanese population

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Summary. The present study demonstrated that a decreased frequency of HLA-BW52 was a common characteristic shared by the patients with Graves' disease and insulin-dependent diabetes mellitus with juvenile onset among Japanese.

It was pointed out that a decreased frequency of HLA-B5 was a common characteristic in patients with Graves' disease and insulin-dependent diabetes mellitus with juvenile onset (JOD)[1,2] among Japanese population. Recently HLA-BW51 and BW52, both splits of B5, were established as new specificities. In the present paper, we shall report the results of HLA typing for BW51 and BW52 in Japanese patients with Graves' disease or diabetes mellitus.

Subjects and methods. 58 Japanese patients with Graves' disease and 69 Japanese patients with diabetes mellitus were HLA typed with the NIH method[3]. 79 healthy controls were also HLA typed. None of them is related to each other, and all of them are living in the Kagoshima area, the southernmost part of Japanese mainland. According to Cudworth and Woodrow[4], the patients developing clinical disease of diabetes mellitus before the age of 30 were regarded as juvenile onset, and those who developed the disease after this age as maturity onset (MOD). Of the diabetics examined 40 patients had JOD. The remaining 29 were MOD patients.