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.

2 J.E. Till, E.A. McCulloch and L. Siminovitch, Proc. nat. Acad. Sci. *51*, 29 (1964).
3 J.E. Till and E.A. McCulloch, Radiat. Res. *14*, 213 (1961).
4 D. Metcalf and M.A.S. Moore, Haemopoietic Cells, p.31 and 493. North Holland, Amsterdam 1971.
5 E.A. McCulloch, L. Siminovitch and J.E. Till, Science *144*, 844 (1964).
6 D.E. Harrison and C.M. Astle, Transplantation *22*, 42 (1976).
7 E.S. Russell and S.E. Bernstein, in: Biology of Laboratory Mouse, p.351. Ed. E.L. Green. McGraw-Hill, New York 1966.
8 L. Siminovitch, E.A. McCulloch and J.E. Till, J. cell. comp. Physiol. *62*, 327 (1963).
9 M.A. Maloney, M.J. Dorie, R.A. Lamela, Z.R. Rogers and H.M. Patt, J. exp. Med. *147*, 1189 (1978).
10 W. Wiktor-Jedrzejczak, S.J. Sharkis, A. Ahmed, K.W. Sell and G.W. Santos, Science *196*, 313 (1977).
11 S.J. Sharkis, W. Wiktor-Jedrzejczak, A. Ahmed, G.W. Santos, A. McKee and K.W. Sell, Blood *52*, 802 (1978).
12 N.S. Wolf and J.J. Trentin, J. exp. Med. *127*, 205 (1968).
13 S. Abramson, R.G. Miller and R.A. Phillips, J. exp. Med. *145*, 1567 (1977).
14 W. Wiktor-Jedrzejczak, S.J. Sharkis, A. Ahmed, A. McKee, G.W. Santos and K.W. Sell, Exp. Hemat. *5*, suppl.2, 36 (1977).
15 R. Schofield, Blood Cells *4*, 7 (1978).

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

A. Kawa, S. Nakamura, Y. Kono, Y. Maeda and T. Kanehisa

The First Department of Internal Medicine, Kagoshima University Medical School, Kagoshima 890 (Japan), 15 August 1978

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.

Table 1. Phenotype frequencies of HLA-BW51 in Japanese patients with Graves' disease or diabetes mellitus

	Control	Graves	JOD	MOD
(+)	4	9	1	3
(−)	75	49	39	26
χ^2		4.265	0.456	0.935
p		<0.05	NS	NS
Corrected p		NS		

Table 2. Phenotype frequencies of HLA-BW52 in Japanese patients with Graves' disease or diabetes mellitus

	Control	Graves	JOD	MOD
(+)	22	1	1	8
(−)	57	57	39	21
χ^2		16.213	10.853	0.023
p		<0.001	<0.001	NS
Corrected p		<0.002	<0.002	
			$\chi^2=9.241$	

Results. Table 1 shows the phenotype frequency of HLA-BW51 in Japanese patients with Graves' disease or diabetes mellitus and the controls. The frequencies of this antigen were 15.5% and 5.1% in the patients with Graves' disease and the healthy controls, respectively. The frequency of BW51 in the patients with Graves' disease showed a tendency to increase, but this was not statistically significant following correction for the number of antigens (corrected $p < 0.10$). There was no difference in the frequencies of this antigen among JOD patients, MOD patients and the controls.

Table 2 demonstrates the frequencies of HLA-BW52 in the patients and the controls. The frequencies of BW52 were 1.7% and 27.8% in the patients with Graves' disease and the healthy controls, respectively. Thus the frequency of this antigen in the Japanese patients with Graves' disease was significantly lower than that in the controls (corrected $p < 0.002$). The frequency of BW52 was significantly decreased also in JOD patients as compared with the controls (corrected $p < 0.002$) or with MOD patients (corrected $p < 0.02$). There was, however, no significant difference between MOD patients and the controls.

Discussion. To our knowledge, no report has been presented so far concerning the frequencies of HLA-BW51 and BW52 in Japanese patients with Graves' disease and JOD. The present study demonstrated that a decreased frequency of HLA-BW52 is a common characteristic shared by Japanese patients with Graves' disease and JOD. This finding seems to be noteworthy, since there is ample evidence that, in both conditions, abnormalities in immune responses play an important role in the development of the diseases. Though it is well-known that among Causasians an increased frequency of B8 is a common characteristic of the HLA phenotype in patients with Graves' disease[5] and JOD[6], there is no such common antigen(s) in the case of a decreased frequency. It appears noteworthy that among Japanese there is no common characteristic in the 2 endocrine diseases mentioned above as far as an increased frequency is concerned[1,2], but a decrease in the frequency of BW52 is found in both Graves' disease and JOD. The clinical implication of this finding is still to be elucidated, since no report has been presented concerning the relation between immune capacities and BW52 in the Japanese population.

1 A. Kawa, S. Nakamura, M. Nakazawa, S. Sakaguchi, T. Kawabata, Y. Maeda and T. Kanehisa, Acta Endocr. *86*, 754 (1977).
2 A. Kawa, M. Nakazawa, Y. Kono, S. Sakaguchi, S. Nakamura and T. Kanehisa, Experientia *34*, 669 (1978).
3 P.I. Terasaki, B. McCurdy and J. McClelland, in: Manuals of Tissue Typing Techniques, p.67. Ed. J.G. Ray. TIB, NIAD, NIH, Bethesda 1974.
4 A.G. Cudworth and J.C. Woodrow, Diabetes *24*, 345 (1975).
5 F.C. Grumet, R.O. Payne, J. Konishi and J.P. Kriss, J. clin. Endocr. *39*, 1115 (1974).
6 J. Nerup, P. Platz, O. Ortved Andersen, M. Christy, J. Lyngsøe, J.E. Poulsen, L.P. Ryder, L. Staub Nielsen, M. Thomsen and A. Svejgaard, Lancet *2*, 864 (1974).

Preparative separation of nucleated cells from human bone marrow[1]

J.J. Minguell, L. Bolton, R.E. Helmer III and F.H. Gardner

University of Texas Medical Branch, Division of Hematology/Oncology, Galveston (Texas 77550, USA), 24 July 1978

Summary. A comparison has been made between the separation of nucleated cells from human bone marrow aspirates by high mol. wt polymers and the buffy coat techniques.

Cell separation is a critical problem for the study of hormone response on bone marrow cells when many different precursor types are present. One may expect in bone marrow aspirates a marked dilution of the nucleated cells by red cells. To reduce erythrocyte contamination aqueous solutions of high mol. wt polymers such as dextran, ficoll, and albumin have been used for a more effective nucleated cell separation[2,3]. A comparison has been made between the separation of nucleated bone marrow cells by high mol. wt polymers technique, and the collection of nucleated cells by centrifugation and separation of the buffy coat. While the buffy coat method is not as effective in the removal of red cells, it does provide a better yield of viable nucleated cells.

Materials and methods. Human bone marrow aspirates (3 ml), diluted with sterile phosphate-buffered saline (PBS) (10 ml) containing 167 U/ml of beef heparin were carefully passed through 19 and 23 gauge needles. The cell suspension after filtration through gauze represents the whole bone marrow (WBM). A suspension of WBM (400×10^6 cells/ml) was mixed with Dextran 75 in 0.9% saline (Abbott Lab.) in a ratio of 5:1 and allowed to settle on ice for 30 min. The suspended cells were removed and washed twice with PBS. A WBM suspension or a post-Dextran cell-PBS suspension (400×10^6 cells/ml) were layered over Ficoll-Paque solution (Pharmacia) in a ratio of 5:4 and spun at $400\times g$ at 12 °C for 40 min. The cells at the interface were harvested and washed twice with PBS.

When the buffy coat method was used, WBM cells in PBS were spun at $600\times g$ at 10 °C for 10 min. The cell pellet was resuspended in PBS and pipetted into a Kolmer buffy coat tube and spun at $1800\times g$ for 10 min at 10 °C. The buffy