processed for EM by the method of White[9]. Samples were sectioned and then stained with lead citrate and uranyl acetate.

Results. Antisickling effect of BAPB: Phase-contrast microscopy showed that 95% of the cells treated with 5×10^{-3} M BAPB, after deoxygenation, were not sickled. In contrast, 95% of the untreated cells were sickled after deoxygenation. SEM studies confirmed this finding and representative micrographs of treated and untreated cells are shown in figure 2.

Table 1 shows that the maximum antisickling effect of BAPB was achieved at a concentration of 5×10^{-3} M or higher. Table 2 shows that BAPB-treated hemolysates gel at higher hemoglobin concentrations and have longer delay times of gelation than those of untreated hemolysates.

Ultrastructure: TEM micrographs of gels of untreated deoxygenated hemolysates of S/S blood were similar to each other with masses of filamentous rodlike structures present. Gels of BAPB-treated, deoxygenated hemolysates of S/S blood were distinctly different and showed a lack of discernable filamentous structure. Representative micrographs of BAPB-treated gels (A) and untreated gels (B) are shown in figure 3.

Discussion. The studies reported here indicate that BAPB is a potent antisickling agent and possibly acts by preventing the polymerization of hemoglobin S. BAPB inhibits in vitro sickling of S/S Erythrocytes and inhibits gelation of hemoglobin. BAPB-treated gels of hemoglobin S do not show the presence of rod-like hemoglobin fibres and resemble the pictures of gels of oxygenated hemoglobin S[8,10]. The increase in delay time of gelation and ultrastructural appearance of gels of BAPB-treated hemoglobin indicate that BAPB reacts with hemoglobin.

The breakdown products of BAPB are 2-{benzoylamino} pyridine and benzoic acid[11] (figure 1). Neither of these products have shown any antisickling activity in in vitro studies[2]. It has been found that BAPB is far more lipophilic than either of its 2 component parts and we presume that the lipophilic nature of BAPB allows it to pass through the membrane into the cell where it or its breakdown products reacts with hemoglobin S by carbamylation. Studies are presently being conducted to further define its mechanism of action.

1 Acknowledgments. This research has been partly funded by a Sickle Cell Research grant from the State of Tennessee to Meharry Medical College. We gratefully appreciate the technical assistance of Mr Rudolf Harlan and Mrs Marjorie Deveau of the School of Graduate Studies. We offer our thanks to Dr J.W. Davis, J.D., Coordinator, Sickle Cell Project, Meharry Medical College for constant encouragement.

2 P.K. Adhikary, A.K. Bhattacharya and A.M. Isa, manuscript in preparation.
3 A. Cerami and J.M. Manning, Proc. natl Acad. Sci. USA *68*, 1180 (1971).
4 C.K. Lee, J. biol. Chem. *25*, 6226 (1976).
5 D.R. Harkness and S. Roth, in: Progress in Hematology, vol. IX, p. 157. E. Grune Stratton Inc., 1975.
6 K. Singer, I.C. Chernoff and L. Singer, Blood *6*, 413 (1951).
7 R.M. Nalbandian, Molecular aspects of sickle cell hemoglobin; American Lecture series, Pub. No. 818:31, Charles C. Thomas. Springfield 1971.
8 J.G. White and B. Heagan, J. exp. Med. *131*, 1079 (1970).
9 J.B. White, Blood *31*, 561 (1968).
10 J.B. White and B. Heagan, Am. J. Path. *58*, 1 (1970).
11 E.H. Huntress and H.C. Walter, J. org. Chem. *13*, 735 (1948).

The environment in which lymphocytes differentiate influences their ability to cooperate in vivo

M. Marušić[1]

The University of Tennessee, Oak Ridge Graduate School of Biomedical Sciences and Biology Division, Oak Ridge National Laboratory[2], Oak Ridge (Tennessee 37830, USA), 19 December 1977

Summary. Whether or not parental B lymphocytes cooperate with F1 T lymphocytes depends upon the environment in which parental bone marrow cells differentiate. Only those parental B lymphocytes that have differentiated in the F1 environment are able to cooperate with F1 T lymphocytes.

The cooperation of T and B lymphocytes in the process of antibody production represents not only an interesting immunological phenomenon but may also serve as a general model of cell interactions and differentiation. It is now well established that cooperation of T and B lymphocytes is controlled by the major histocompatibility complex (MHC) genes[3]. It has been proposed that, in order to cooperate, T and B lymphocytes have to share or complement cell membrane determinants coded by certain MHC genes[3]. However, recent studies suggest that the genetic restriction of T-B cooperation may be a dynamic process dependent on the environment in which the lymphocytes are exposed to antigen. F1 lymphocytes primed in irradiated parental recipients were shown to interact preferentially with the cells of the respective parental haplotype[3,4]. The existence of 'adaptive differentiation' was thus suggested[3,4] proposing that antigen-driven differentiation in a semi-syngeneic environment allows the lymphocytes to interact preferentially with host-type cells.

The present experiment was designed to investigate the concept of adaptive differentiation in a model of immune response of mice to rat Yoshida ascites sarcoma (YAS). YAS grows well in the abdominal cavity of T-cell deficient thymectomized lethally irradiated bone marrow reconstituted (TIR) mice and kills the recipients[5]. Tumor growth can be inhibited by a single injection of normal syngeneic T lymphocytes[6]. Tumor rejection was shown to be due to antibody production involving interaction of transferred T and host B lymphocytes[6]. The failure of allogeneic cells to cooperate has been demonstrated in this model for normal lymphocytes[6,7] as well as for lymphocytes from mutually tolerant long-term radiation chimeras[7]. Both P→F1 and normal parental T lymphocytes cooperated with F1 B lymphocytes. However, F1 T lymphocytes failed to cooperate with parental B lymphocytes but did cooperate with parental B lymphocytes from P→F1 radiation chimeras[7]. This difference in the ability of P→F1 chimeric and normal parental B lymphocytes to cooperate with F1 T cells[7] constituted a basis for the present study. TIR mice of P→P, P→F1, P→F1→P, P→F1→F1, and F1→F1 constitutions were injected with YAS cells and with F1 T lymphocytes. The rejection of the tumor indicated a suc-

Rejection of YAS in TIR mice given splenic T lymphocytes*

T-cell donor	Deaths/total number of TIR recipient mice of strain constitution: B6→B6D2F$_1$→B6	B6→B6D2F$_1$→B6D2F$_1$	B6→B6D2F$_1$	B6→B6	B6D2F$_1$→B6D2F$_1$
None	9/12	8/9	7/7	8/10	7/8
B6	2/13	2/10	1/8	1/10	ND**
D2	9/10	10/10	6/6	9/10	3/8
B6D2F$_1$	12/14	0/14	0/7	8/10	0/6

* Thymectomized, lethally irradiated mice were reconstituted with 4×10^6 anti-Thy 1.2-antiserum-treated bone marrow cells (TIR mice). 2 months later they were injected i.p. with 30×10^6 YAS cells, and 1 day after that i.v. with 2×10^6 nylon-wool-nonadherent spleen cells. Mortality was scored for 2 months. Rejection of the tumor was considered a successful cooperation of donor T and recipient B lymphocytes. ** Not done (because B6 spleen cells kill B6D2F$_1$ TIR mice)[7].

cessful cooperation of F1 T and parental B lymphocytes. The purpose of the experiment was to explore whether or not the retransfer of bone marrow cells from P→F1 chimeras back to parental strain mice (in the absence of the exogenous YAS antigens) would reverse the ability of the parental B lymphocytes to cooperate with F1 T lymphocytes.

Materials and methods. Mice of C57BL/6 (B6) and DBA/2(D2) strains and hybrids of (C57BL/6♀X DBA/2♂)F1(B6D2F1) constitution were purchased from Jackson Memorial Laboratory (Bar Harbor, ME). Mice were thymectomized at 6–8 weeks of age and were irradiated 4 weeks later. B6 mice received 850R and B6D2F1 mice 950R of X-rays. Normal B6 and B6D2F1 mice and B6→B6D2F1 long-term (4 months) radiation chimeras served as bone marrow donors. Bone marrow cells used for reconstitution of irradiated mice (4×10^6 per animal given i.v.) were pretreated with anti-Thy 1.2 antiserum and complement. TIR mice were used in the experiment 2 months after irradiation and reconstitution. Their chimerism was analyzed by erythrocyte typing 3–5 days before they were injected with the YAS. The agglutination[8] and the lysis methods[9] were used in parallel. At least 50% of the chimeras of each constitution were individually analyzed. In no case did these tests reveal anything but donor cells. The method described by Julius et al.[10] was used to separate splenic T lymphocytes. The resulting population of nylon-wool-nonadherent small lymphocytes contained 75–85% T and 1–5% B lymphocytes as judged by immunofluorescence staining.

Results and discussion. TIR mice of B6→B6D2F1→B6, B6→B6D2F1→B6D2F1, B6→B6 and B6D2F1→B6D2F1 strain constitutions were injected i.p. with 30×10^6 YAS cells and, 1 day later, i.v. with 2×10^6 nylon-wool-nonadherent spleen cells from B6, D2, or B6D2F1 donors. YAS-challenged but nonlymphoid-cell-injected controls were always included. Mortality was scored daily for 2 months. TIR mice of all strain constitutions that had not been injected with lymphoid cells succumbed after YAS injection. B6 splenic T lymphocytes induced YAS rejection in TIR mice of all used constitutions. D2 splenic T lymphocytes failed to promote YAS rejection in B6→B6D2F1→B6, B6→B6D2F1→B6D2F1, B6→B6D2F1, and in B6→B6 mice thus confirming our earlier report on inability of allogeneic T and B lymphocytes to cooperate in this model[7]. The survival rate of B6D2F1 TIR mice given D2 splenic T lymphocytes was not significantly different from either that of B6D2F1 TIR mice given no T cells or from that of B6D2F1 mice given B6D2F1 T cells (tested by χ^2 test with Yates correction). However, the rejection of the tumor in 5 of 8 animals probably reflects the cooperation of parental donor T and F1 host B lymphocytes as we have reported earlier[7]. B6D2F1 splenic T lymphocytes helped syngeneic B6D2F1→B6D2F1 TIR mice to reject YAS and also provided help to B6→B6D2F1 and B6→B6D2F1→B6D2F1 TIR recipients, but did not influence progressive tumor growth in B6→B6D2F1→B6 or B6→B6 recipients.

These results indicate that in the immune response of mice against YAS F1 T lymphocytes can cooperate with parental B lymphocytes only if the latter have differentiated it the F1 environment. Inasmuch as the F1 lymphocytes cooperated with parental B lymphocytes from P→F1 and P→F1→F1 mice but failed to cooperate with B lymphocytes from P→F1→P mice, it is apparent that the F1 environment did not change undifferentiated parental bone marrow stem cells but that the differentiation of these cells into functional B lymphocytes was altered allowing them to cooperate with F1 T lymphocytes. Because it was possible to reverse the ability of parental B lymphocytes to cooperate with F1 T lymphocytes before YAS injection (i.e., needing only the retransfer of B6 bone marrow cells from B6→B6D2F1 mice to B6 mice), it appears that this ability of parental B lymphocytes to cooperate with F1 T lymphocytes was affected solely by the differentiation of the former in the F1 environment and was independent of the presence of the exogenous (YAS) antigen.

1 Postdoctoral Investigator supported by Subcontract No. 3322 from the Biology Division of Oak Ridge National Laboratory to the University of Tennessee and by the Fulbright-Hays Program. Present address: Department of Physiology, University of Zagreb Faculty of Medicine, Zagreb, Yugoslavia.

2 Operated by the Union Carbide Corporation for the Department of Energy.

3 B.J. Skidmore and D.H. Katz, J. Immun. *119*, 694 (1977).
4 D.H. Katz and B. Benacerraf, in: The role of the products of the histocompatibility gene complex in immune responses, p. 355. Ed. D.H. Katz and B. Benacerraf. Academic Press, New York 1976.
5 M. Marušić, N. Allegretti and F. Čulo, Int. Archs. Allergy appl. Immun. *49*, 568 (1975).
6 M. Marušić, N. Allegretti and F. Čulo, Cell. Immun. *27*, 26 (1976).
7 M. Marušić, J.W. Goodman and S.G. Shinpock, Cell Immun. *33*, 72 (1977).
8 P.A. Gorer and Z.B. Mikulska, Cancer Res. *14*, 651 (1954).
9 R.D. Owen, in: Biological problems of grafting, p. 260. Ed. F. Albert, Blackwell Scientific, Oxford 1959.
10 M.H. Julius, E. Simpson and L.A. Herzenberg, Eur. J. Immun. *3*, 645 (1973).