Bone-Marrow Studies in Thai Haemorrhagic Fever *

SUPA NA-NAKORN, ANONG SUINGDUMRONG, SANGA POOTRAKUL & NATTH BHAMARAPRAVATI

Bone-marrow studies on 29 virologically proven cases of Thai haemorrhagic fever are reported on in this communication.[1] The clinical material was obtained from patients from the Thai Haemorrhagic Fever Study Centre and Siriraj Hospital, Bangkok. The age of the patients ranged from 11 months to 16 years, averaging 13 years. All had dengue virus infection but four were also infected with chikungunya virus. The bone-marrow aspirations were done twice on five of the patients, so that, in all, 34 marrow specimens were obtained.

Preparations were made from direct smears of the aspirated marrow particles in all cases, and in 10 of them paraffin sections, with haematoxylin-eosin staining of the aspirated marrow particles, were also prepared.

The patients were divided into two groups: thrombocytopenic and non-thrombocytopenic. The thrombocytopenic group, consisting of 21 patients, was further divided into three subgroups, according to the stage of the disease—namely, prethrombocytopenic, thrombocytopenic and recovery.

Bone marrow was obtained from five patients in the *prethrombocytopenic stage* during the second to fourth days of illness; all the patients went on to develop thrombocytopenia on the fourth to eighth day. The cellularity was moderately decreased in all cases. Erythroid cells were diminished in number in all cases, some with arrest of maturation. The granulocytes were decreased in number and showed a shift to the myelocytic stage. The megakaryocytes were normal in number and appearance in all cases, but younger forms were present in some. There was a definite increase in the number of small lymphocytes, reticulum cells, phagocytic clasmatocytes and monocytes. There were vacuoles in some of the granulocytes, monocytes and megakaryocytes. Plasma cells were slightly increased in one case.

In the *thrombocytopenic stage*, the bone marrow was obtained during the fourth to eighth days of illness from 16 patients with disease of various degrees of severity. The cellularity was increased in most of the cases, owing to erythroid hyperplasia with arrest of maturation. Erythroid cells resembling those of the megaloblastic series of pernicious anaemia were noticed in five cases.

In 13 cases megakaryocytes were increased in number, and consisted of a mixture of mature and young forms without platelet around; the remainder of the patients had a normal number of megakaryocytes. Other findings were similar to those in the previous stage.

In the *recovery stage*, bone marrow was obtained from six cases during the tenth to fourteenth days of illness, when the platelets had returned to normal or there was thrombocythaemia. The cellularity was moderately increased in all cases, owing to erythroid hyperplasia without arrest of maturation. The megaloblasts were still present. Megakaryocytes of both mature and immature forms were moderately increased in number, all showing very active production of platelets. The other cellular elements were normal, except for exhibiting moderate lymphocytosis.

Bone marrow was obtained from seven cases in the *non-thrombocytopenic group* during the fourth to ninth days of illness. Platelet counts were normal, but all patients had petechiae and a positive tourniquet test. The cellularity was normal or slightly increased. The megakaryocytes were normal in three cases, but increased in four, consisting of mature and young forms. The lymphocytes were increased. Vacuoles were present in myelocytes and megakaryocytes. The erythroid cells were normal, with arrest of maturation in some cases.

Platelet agglutinin was positive in 6 cases out of 22. On repeated testing, the platelet agglutinin disappeared in the recovery phase.

Evidently the bone marrow is another target organ in Thai haemorrhagic fever. Injury is manifest early in the course of the illness by a reduction in cellularity and the presence of vacuoles in various cell types. This reversible bone-marrow damage could

be induced by the virus itself, or by the resultant toxic products of viral infection, or by an immunological process. In favour of the last-mentioned are the increase in the number of lymphocytes in the bone marrow with circulating plasmacytoid atypical lymphocytes, the detection of platelet agglutinin in some cases and the megakaryocytic picture resembling that in idiopathic thrombocytopenic purpura and St Louis encephalitis. In the recovery phase there was overcompensating thrombocytopoiesis, as evidenced by megakaryocytic hyperplasia, with an

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increased number of platelets at the periphery and thrombocythaemia.

Arrest of maturation of the erythroid cells in the prethrombocytopenic phase and megaloblastic metaplasia in the thrombocytopenic phase indicate a disturbance in nucleic acid synthesis. This could be due to a relative folic acid deficiency, induced by overactive erythropoiesis, particularly if there was pre-existing latent folic acid deficiency. Another possibility is that the virus RNA itself acts as a nucleic acid inhibitor in the erythroblasts.

Immunological Response: Possible Role of Human Response as an Etiological Factor *

W. M. HAMMON [1]

Several hypotheses may be presented to explain why viruses in the dengue group appear to produce on the one hand a benign febrile disease called dengue and on the other a very different and serious haemorrhagic disease. One hypothesis is that there are two kinds of dengue viruses. A second is that host factors play the all-important role. This paper deals with one aspect of the second hypothesis.

Immunological sensitization can explain many types of pathology and could conceivably produce the type of pathology and symptomatology observed in haemorrhagic fever. This is the hypothesis. The evidence presented for and against it will be of a serological and epidemiological nature, and not experimental.

The assumption is that one or several previous dengue or closely related virus infections are prerequisite for sensitization for the haemorrhagic disease, which may occur with the next exposure to a dengue virus of still another antigenic type. Sabin [2] demonstrated that a type 1 or type 2 dengue infection

will modify a following heterologous type infection if the time interval is short enough, and also showed that quite solid immunity is present for the homologous type. The fact that adult residents of endemic areas have serum antibodies reacting *in vitro* to all types of dengue viruses and many other group B viruses and do not become ill during haemorrhagic fever epidemics is evidence that following a number of dengue virus infections essentially complete immunity occurs. The hypothesis is that after several infections, before complete immunity has occurred, there is a critical period of sensitization. Foreigners and short-term visitors are unlikely to be sensitized.

This hypothesis was proposed to explain why haemorrhagic fever was observed only in native Orientals in Manila, Bangkok and Singapore, yet classical dengue was recognized at the same time in short-term foreign residents. Why should two different types of viruses carried by the same vector select their hosts in such a discriminating manner? Of course, other host factors needed equal consideration but no strong case could be presented for any. There seemed little support for any obvious genetic factor, for the epidemics were new and endemic dengue and the several races were not. Dietary changes—excesses or deprivations—would have to be newly introduced and occur almost simultaneously in all epidemic areas and not in many nearby areas where dengue virus was endemic but not haemorrhagic fever. Sensitization might be new, if recently increased transportation, particularly

[2] Sabin, A. B. (1950) *Bact. Rev.*, 14, 225.