BLOOD FORMATION IN INFANCY

PART I. THE NORMAL BONE MARROW

BY

DOUGLAS GAIRDNER, JOHN MARKS and JANET D. ROSCOE

From the Cambridge Maternity Hospital and the Department of Pathology, University of Cambridge

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A complete account of the changes in the blood picture in infancy requires a knowledge of the rates of formation and destruction of blood. Since the latter is not easily measured, our approach has been to use the activity of bone marrow as a measure of blood formation, and then to deduce whether changes in blood formation alone are sufficient to account for observed changes in the blood picture. Part I of the study, therefore, consists of an account of the marrow picture during the first three months of life. Part II concerns erythropoiesis, and the marrow findings are then considered together with the blood picture.

Material and Methods

The data to be discussed are derived from a total of 102 specimens of marrow from 25 infants aged from a few minutes to three months. A sample of venous blood was taken at the same time as the marrow.

The infants were born in the Cambridge Maternity Hospital; the majority were chosen because their mothers had already borne a normal infant at the hospital and had volunteered to take part in the investigation; three subjects were mongols, but as their weight gains proved normal during the period under review, they were included in the series. Labour and delivery were normal, minimal nitrous oxide and air anaesthesia being given to the mothers. Birth weights exceeded 6 lb. The cord was clamped as soon as conveniently possible, but usually some 10 to 15 seconds elapsed before this was done. Infants developing more than trivial infections were excluded. Sixteen of the 25 infants were completely breast fed. Weight gains were satisfactory and the infants generally were healthy and well cared for.

Samples of marrow and venous blood were taken when possible at the following ages: birth, first day, 3-5 days, 8-10 days, 1 month and either 2 or 3 months. Not every infant completed the full two or three months, hence there are more data for the first month than for later, but 14 infants were followed for the full two to three months.

Marrow Samples. These were obtained by tibial puncture using the technique and special needles described by one of us (J.D.R.) (Gimson, 1944). The procedure takes no longer than a venipuncture and no anaesthetic was used. The method has been used on about 250 occasions in young infants, with very rare failure to obtain a good sample of marrow. After trying other sites, sternum, ilium, vertebra and femur, we have found tibial puncture the most reliable method for young infants: 0·25 ml. of marrow was aspirated, smears made, and the remainder mixed with Wintrobe's oxalate mixture (Wintrobe, 1946). From the oxalated marrow total nucleated cells were counted in a white cell chamber. A differential cell count of 200 cells was performed on the marrow films. Smear cells were counted separately (see below). The nomenclature given by Whitby and Britton (1950) has been used throughout.

Venous Blood. This was obtained from the internal or external jugular vein and mixed with Wintrobe's oxalate mixture. Cord blood was obtained by umbilical vein puncture after clamping the cord. Haemoglobin was determined as oxyhaemoglobin in a photoelectric colorimeter. The blood dilution was 1 in 200, using 0·04% ammonia as diluent. The colorimeter was checked weekly against the Medical Research Council type grey wedge standard photometer (Duffie, 1945). A further check of both instruments was made monthly with iron determinations on several blood samples. Erythrocyte and leucocyte counts were made by conventional methods, counting 0·005 c.mm. of blood for the red cells, and 0·04 c.mm. of blood for the leucocytes. Differential leucocyte counts were performed on films made with an automatic slide-making machine (Marks, Bailey and Gunz, 1950), counting 200 cells by the battlement technique (MacGregor, Richards and Loh, 1940). Packed cell volume was estimated in a Wintrobe haematocrit tube, spun for 30 minutes at 3,000 r.p.m. Reticulocytes were counted by a modification of the Della Vida technique after counterstaining with Leishman, a preliminary investigation having shown that this gave reproducible results: one thousand red cells were enumerated, a number insufficient to give results of great accuracy, but sufficient to show the large

alterations in reticulocyte level which occur in infancy.

Total Circulating Haemoglobin and Red Cells. In view of the rapidity of body growth in infancy, concentrations of blood cells may give a misleading idea of alterations in their absolute number, and it has proved convenient to estimate the total number of the various types of cells in the circulation from the product of their concentration and the blood volume. These have been derived as follows. The observed concentrations of haemoglobin and red cells in venous blood have been multiplied by the factor 0·87 to allow for venous blood having a higher concentration of red cells than has the circulating blood as a whole (Mollison, Veall and Cutbush, 1950). No such correction has been applied to the white cells since, in the absence of information on this point, we have assumed that their concentration in venous blood is representative of the blood as a whole. Blood volume has been calculated from the data recently published by Mollison *et al.* (1950) and Mollison (1951) where blood volume is shown as a function of body weight and 'venous haematocrit'. Mollison derives 'venous haematocrit' from the observed packed red cell volume (P.C.V.) by the formula (P.C.V.—2·0) × 0·95. The factor 0·95 is a correction to allow for plasma trapped in the red cell layer. The 2 units subtracted from P.C.V. in the formula allows for the difficulty in reading the top of the red cell layer precisely when working with blood from young infants, owing to the red and white cell layers sometimes merging indefinitely. We have observed the same phenomenon, but only in blood of infants in the first day of life, and have then attempted to allow for it by taking as the top of the red cell layer a point half way up the indefinite zone. For this reason we have preferred to take 'venous haematocrit' as simply P.C.V. × 0·95. The blood volume obtained by Mollison and his colleagues were derived data from infants less than 24 hours old, but they give cogent reasons for believing that they apply equally to adults, hence it seems reasonable to apply them to older infants, and this we have done, considering that no more accurate figures are available. However, inspection of the curve connecting blood volume and venous haematocrit (Mollison, 1951) shows that blood volume begins to increase to an important degree with increasing haematocrit only when the latter reaches high levels. It happens that our main conclusions have not depended upon observations involving these high haematocrits, and hence, had we chosen to compute blood volumes as a fixed proportion of body weight, this would have made no significant difference to these conclusions.

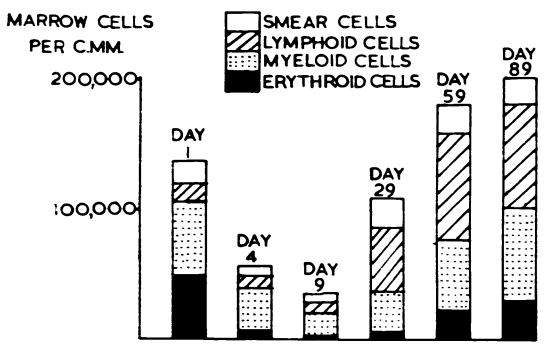

FIG. 1.—The cellular pattern of the bone marrow from birth to 3 months. (The small number of monocytes is not shown.)

Results and Discussion

Since the data on marrow refer to tibial marrow, conclusions drawn from this would only be valid if the red marrow throughout the body could be considered homogeneous. This uniformity has been found in adults by Stasney and Higgins (1939) who examined marrow from the sternum ribs, vertebrae and femora of 14 subjects after accidental death by Rubinstein, (1948), who compared 216 marrows from iliac crest and sternum, and by Leitner (1949) who punctured sternum, ribs and vertebrae in 10 cases. In children Lamy, Sée, Chiche and Montefiore (1939) obtained similar results on the sternal and tibial marrow up to 8 years of age but give no details. Traina and Velasco (1949) studied the marrow of sternum, tibia, femur and vertebrae in 12 infants who presented a normal peripheral blood picture and could find no significant differences between samples from different puncture sites, while Lato (1947) could find no difference between marrow from sternum, tibia and iliac crest in either healthy or diseased children.

To confirm these findings in the neonatal period, we have performed a number of multiple marrow examinations.

(1) Histological sections were examined from several bones of each of 10 infants who had died soon after birth or had been stillborn. Marrow from the different sites showed no significant differences in total cellularity or in relative numbers of the different marrow cells.

(2) In seven infants dying shortly after birth, three from obstetric causes and four from haemolytic disease, marrow punctures were made from three or more sites—sternum, femur, iliac crest or tibia. The different sites showed a uniformity both of total cellularity and differential count.

(3) Multiple marrow punctures were performed on four live infants. Marrow from different sites showed no major differences of total cellularity or differential count (Table 1).

Total Nucleated Cell Count. In 94 samples a total nucleated cell count was performed. In the remaining eight insufficient marrow was obtained for a count and the cellularity was estimated from the stained smear, for with experience it was found that the total nucleated cells could be estimated

TABLE 1

CELL COUNTS OF MARROW SAMPLES FROM TWO OR MORE SITES*

	Case 1		Case 2		Case 3		Case 4		
Age	4 days		11 days		28 days		5 days		
Site	Left Tibia	Right Tibia	Sternum	Right Tibia	Sternum	Left Tibia	Sternum	Left Tibia	Right Tibia
Total nucleated cells (per c.mm.)	24,000	31,000	100,000	80,000	51,000	40,000	75,000	45,000	50.000
Erythroid cells (%) ..	10·5	11·5	1·00	1·5	5·0	3·0	19·5	15·0	10·0
Myeloid cells (%) ..	79·0	74·0	88·5	77·5	55·5	41·0	75·0	76·5	86·0
Lymphoid cells (%) ..	10·0	14·5	7·0	16·0	47·5	53·0	5·5	7·5	3·5
Monocytes (%) ..	0·5	—	3·5	5·0	2·0	3·0	—	1·0	0·5

* Note that samples from different sites show fair agreement.

with moderate accuracy. Since these eight counts were distributed almost uniformly throughout the age period no great loss of accuracy would result from this. The total nucleated cell count has generally been considered of little significance on account of the unknown degree of dilution of marrow with blood in the aspirate. In our experience with both normal and anaemic infants, however, the nucleated cell count has been reasonably uniform when samples from different sites are compared (Table 1).

Fig. 1 and Table 2 show that there is a considerable fall in the total nucleated cell count over the first 10 days, from 136,000 to 35,000 per c.mm., followed by a rise to above adult values from four weeks (108,000) to three months (201,000). These figures could be explained either on the basis of blood admixture occurring more readily at the fourth and ninth day punctures, or of a genuine difference in the sample. Blood dilution affecting particularly these two counts is unlikely. Furthermore, examination of the differential counts (Table 3)

TABLE 2

BONE MARROW FROM BIRTH TO 3 MONTHS

Number of Cases	0–24 hours 19		3–5 days 23		8–10 days 23		26–33 days 19		55–62 days 6		82–99 days 12	
Total nucleated cells (thousands per c.mm.)	136	+(45-290)	56	(5-117)	35	(5-100)	108	(61-248)	180	(62-338)	201	(108-449)
Smear cells (per 100 cells that could be named)	14·6	(1-43)	13·4	(0-40)	19·6	(0-40)	25·3	(0-80)	16·1	(0-41)	14·0	(0-50)
Named cells (thousands per c.mm.)	121	(41-250)	50	(5-117)	30	(4·2-85)	91	(6-228)	157	(44-296)	186	(95-429)
Mitotic figures in normoblasts per 100 nucleated cells ..	0·49	(0-1·5)	0·22	(0-1·0)	0·01	(0-0·5)	0·14	(0-0·5)	0·38	(0-1·5)	0·19	(0-1·0)
Erythroid cells (%)* ..	40·0	(18·5-65)	15·3	(0-32)	8·0	(0-20·5)	6·8	(2·0-16)	14·8	(7·5-34)	16·0	(6·5-31·5)
Myeloid cells (%)* ..	46·4	(20-73)	60·6	(44-88)	50·0	(34-73)	31·1	(13-55)	36·6	(22-50·5)	36·8	(10-86)
Lymphoid cells (%)* ..	12·1	(2·0-22·5)	22·9	(7·5-58)	37·3	(20-64)	55·6	(43-84)	48·5	(15-61)	47·0	(4·5-83·5)
Monocytes (%)* ..	1·5	(0-3·0)	1·2	(0-3·0)	1·7	(0-3·0)	1·1	(0-2·5)	0·1	(0-0·5)	0·5	(0-2·0)
Erythroid cells (thousands per c.mm.)	48·6	(13-124)	6·99	(0-24)	2·8	(0-9·2)	6·3	(0·18-19)	22·3	(4·6-50·4)	29·5	(6·9-83)
Myeloid cells (thousands per c.mm.)	56·3	(17-108)	33·3	(1·7-79)	18·2	(3·3-62)	29·0	(4·3-82)	53·9	(15-97)	71·8	(11-172)
Lymphoid cells (thousands per c.mm.)	14·2	(2-44)	9·59	(1·8-30)	9·2	(2·2-27)	54·9	(4·4-162)	81·0	(15-181)	79·7	(9-135)
Monocytes (thousands per c.mm.)	1·9		0·6		0·4		0·8		0·1		0·1	

\+ The mean of each group is given, with the range in brackets.
* The detailed distribution of the different types of cells is given in Table 3.

shows that the proportion of immature cells of the myeloid series is virtually unchanged, although admixture of blood large enough to cause these total changes would lead to a relative increase in the more mature myeloid cells at the expense of the primitive ones. A smaller but still significant drop of this kind was found by Shapiro and Bassen (1941).

From these considerations it is clear that variations in the total nucleated cell count of the marrow are significant, so that it becomes logical to express the numbers of the different types of cells in terms of their concentrations per c.mm. in the marrow. In spite of the relative inaccuracy of their measurement, since neither the total nucleated cell count nor the differential count is measured with great accuracy, experience with normal and abnormal infants has satisfied us that the best available measure of activity of the different haemopoietic tissues of the marrow, erythroid, myeloid and lymphoid, is provided by the concentrations of these cells in marrow aspirate.

Smear Cells. The presence of large numbers of smear cells in the marrow of normal and abnormal adults and children has been ignored by the standard authorities (Leitner, 1949; Israëls, 1948) probably because no interest has been taken in absolute values of the marrow cells. Examination of infants' marrow showed, however, a markedly variable number of unrecognizable smear cells.

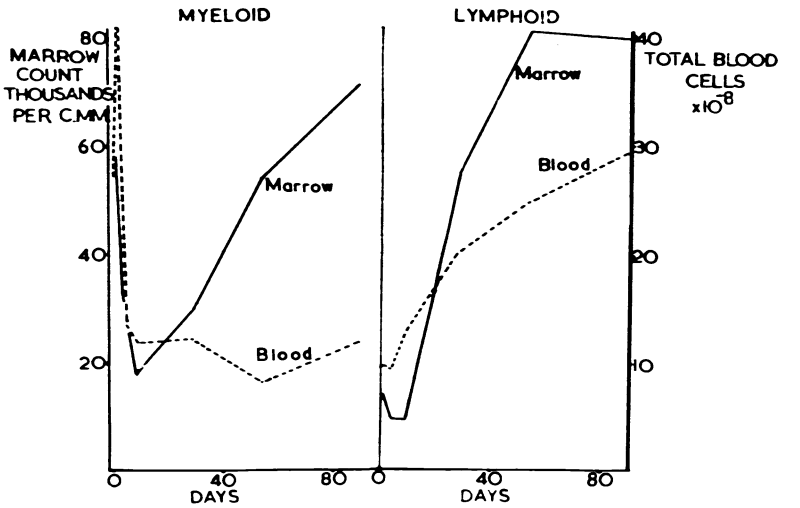

FIG. 2.—The concentration of myeloid and erythroid cells in the marrow, and the total number of myeloid and lymphoid cells in the blood, from birth to 3 months. (For interpretation see text.)

TABLE 3
BONE MARROW FROM BIRTH TO 3 MONTHS

	0–24 hours	3–5 days	8–10 days	26–33 days	55–62 days	82–99 days
Number of cases	19	23	23	19	8	12
Haemocytoblasts	0	0	0	0	0	0
Proerythroblasts	0–1 (0·5)	0–0·5	0	0–0·5	0–1 (0·5)	0–1 (0·5)
Normoblasts, early	0·5–9 (3·0)	0–2·0 (0·5)	0–0·5	0–1·5 (0·5)	0·5–10 (2·0)	0–3·5 (1·5)
,, intermediate	5–19 (10)	0–8·5 (4·0)	0–5 (1·5)	0–3·5 (1·5)	1·5–13 (6·0)	1·0–7·0 (4)
,, late	18–41 (26)	0–23 (10)	0–15 (6)	1–15 (5)	4·5–13 (6·5)	2·5–22·0 (10)
Myeloblasts	0·5–2·0 (1·0)	0–2·5 (10)	0–3 (1·0)	0–2 (0·5)	0·5–5·0 (1·0)	0–4 (1·5)
Promyelocytes	0·5–5·0 (1·5)	0·5–9·0 (3·0)	0·5–7·0 (2·0)	0–4 (1·0)	0–3 (1·0)	1·5–5 (2·0)
Myelocytes, neutrophil	1–9 (4)	3–15 (7·5)	1–11 (4)	1·5–13·5 (4·5)	2–12 (5)	0·5–16 (5)
,, eosinophil	0–2·5 (1·0)	0–2·0 (1·0)	0–2 (1·0)	0–1	0–2 (0·5)	0–2 (0·5)
,, basophil	0–0·5	0–0·5	0–0·5	0	0	0
Metamyelocytes, neutrophil	4·5–25 (14)	4–32 (25)	7–35 (18)	4–31 (10)	3–17·5 (12)	3–33 (11)
,, eosinophil	0–2·5 (1·0)	0–2·5 (1·0)	0–2 (1·0)	0–1	0–2 (1·0)	0–2 (1·0)
,, basophil	0–0·5	0–0·5	0–0·5	0	0–0·5	0–0·5
Polymorphs, neutrophil	10–40 (22)	15–38 (20)	11–45 (20)	2–27 (12)	8–19 (15)	2–24 (15)
,, eosinophil	1·0–3·0 (1·5)	0·5–3·0 (1·0)	0–2 (1·0)	0–1	0–2 (1·0)	0–2 (1·0)
,, basophil	0–0·5	0–0·5	0	0	0	0
Lymphoblasts	0–0·5	0–0·5	0–3·5 (0·5)	0·5–3 (2·0)	0–1·5 (0·5)	0–5·5 (2·0)
Lymphocytes	4–22 (12)	8–36 (23)	20–62 (37)	27–73 (54)	15–60 (48)	31–81 (47)
Plasma cells	0	0	0	0	0	0
Monocytes	0–2·5	0–3 (1·0)	0–3 (1·0)	0–6·5 (1·0)	0–0·5	0–1 (0·5)

* Differential count (percentages). The range is given with average values in brackets.

An average of about 24 of these were present to the standard count of 200 recognizable cells, with individual variations from none to 100. No correlation could be found between the degree of smearing and the amount of suction necessary to withdraw a specimen, the marrow cellularity, or the age of the infant.

This necessarily gives rise to a background of about 5,000-15,000 unrecognizable cells per c.mm. in each specimen. This count seemed to parallel fairly closely the absolute number of lymphocytes present in an individual marrow specimen, and in view of the known fragility of lymphocytes in blood films, it would seem that these smear cells are probably mainly lymphocytes. Since however their nature remains doubtful they have been excluded from further consideration.

The Marrow Differential Count. The range of values for each type of cell and the mean values are given in Table 3. These are published mainly to give a tentative set of values for an age group which has been little dealt with in the literature. Since the number of samples at each age group is not large the range of normals may well be wider than that given.

The cell morphology was identical with that seen in normal adult marrows apart from an occasional macronormoblast in the specimens taken on the first day. No megaloblastic erythropoiesis was seen in any of the marrows examined. The haemoglobinization and nuclear maturation of the normoblasts followed a normal pattern.

ERYTHROID CELLS. At birth the marrow contains on an average 40% erythroid cells. These are rapidly reduced to about 8% during the first 10 days of life. Thereafter they stay relatively constant for about three weeks and then increase to about 15% during the next two months. The striking fall in the proportion of erythroid cells in the first few days of life has been previously noted. Shapiro and Bassen (1941) found a mean of 32% on the first day and 12% on the seventh day for the 35 babies which they examined, agreeing well with our figures; their work was carried out on sternal marrow. Tecilazic (1935) also studied the changes during the first few days of life using tibial marrow: on the first day the erythroid percentage was 66% and on the seventh day 40%, showing a similar but less marked post-natal fall, although the proportions of erythroid cells are much higher than we have found. Lamy *et al.* (1939) conclude that during the first few days of life there are 35%-50% erythroid cells and that during the first week they fall to 25%-35%. Similarly Veeneklaas (1938) records marrow data of a group of three infants under 14 days and finds 12% erythroid cells; although the ages of these infants are not recorded the text suggests an age of between 7 and 14 days. Glaser, Poncher and Limarzi (1948) found a rather low proportion (23%) of erythroid cells during the first two days of life, but agree that there is subsequently a fall followed by a rise.

These changes during the early months of life are seen even more clearly if the total erythroid cells per c.mm. are calculated. These figures show a fall from 46,000 per c.mm. at birth to less than 3,000 per c.mm. at day 9, and then a steady rise from the end of the first month to nearly 30,000 at 3 months (Table 2).

The marrow reticulocytes were also investigated but gave no additional information, for they ran parallel to, but a little higher than, the blood reticulocytes at each age period. The percentage of mitotic figures in the early normoblasts gave a good index of the erythroid activity, for this mitotic activity paralleled the changes in total erythroid count.

The significance of these changes in erythropoietic activity is discussed in detail in Part II of the paper.

MYELOID CELLS. On the first day of life myeloid cells form 46% of the marrow nucleated cells. During the next few days the *percentage* of these cells increases but eventually stabilizes at about 35% of the marrow cells. These findings agree closely with those of Tecilazic (1935), Veeneklaas (1938), Lamy *et al.* (1939), Shapiro and Bassen (1941) and Glaser *et al.* (1948).

The *concentration* of myeloid cells in the marrow falls from birth to day 9 and then rises steadily until the third month. Fig. 2 shows the relationship between the number of myeloid cells in the marrow and in the blood. The latter, expressed as total circulating myeloid cells (Table 4), show a sharp fall after the first day, thereafter remaining at the lower level. Fig. 2 also shows that the fall of the blood myeloid cells after the first day corresponds to the fall in marrow myeloid cells, but that the subsequent rise in marrow myeloid cells produces in the blood no corresponding increase. In a few infants who have been excluded from this series because they developed minor infections, the marrow myeloid cells were increased above normal values although there was no blood leucocytosis. This finding requires further investigation, but it is tentatively suggested that the rise in the marrow myeloid cells is required to provide cells not only for the circulating blood but also for minor inflammatory reactions throughout the tissues of the body.

TABLE 4

TOTAL CIRCULATING LEUCOCYTES FROM BIRTH TO 3 MONTHS (MEANS OF 105 EXAMINATIONS)

	Cord	2–24 hours	3–5 days	8–10 days	26–33 days	55–62 days	82–99 days
Total blood myeloid cells $\times 10^{-8}$	27·2	41·7	13·4	11·6	12·2	8·6	11·7
Total blood lymphoid cells $\times 10^{-8}$	9·3	9·5	8·8	12·7	20·0	24·7	29·3
Total blood monocytes $\times 10^{-8}$	3·3	4·4	2·3	2·7	2·9	1·8	2·7

LYMPHOID CELLS. The marrow lymphoid cells show a steady rise, both in percentage and in number per c.mm., until from the fourth week they form 50% of the marrow cells. This closely parallels the course of total blood lymphoid cells (Fig. 2 and Table 4). Since the concentration of lymphoid cells per c.mm. in the marrow is far greater than in the blood, and since the marrow shows the presence of many lymphoblasts, the marrow must at this stage be a major site for lymphocyte formation, even if this function is largely lost before adult life.

MONOCYTES. We have been unable to confirm the neonatal monocytosis reported by Lamy *et al.* (1939) and in fact have found a steady fall in the total number of monocytes in the blood. The number of monocytes in the marrow also falls over these three months, but these changes are probably not significant on this small number of counts.

Myeloid/Erythroid Ratio. This value has been widely adopted as a measure of myeloid or erythroid activity. In the infant it is of little value, and can in fact be misleading since it takes no account of the large changes which occur both in the total nucleated cell count and in the proportion of lymphocytes.

Summary and Conclusions

The normal marrow picture from birth to 3 months has been defined by examining 102 specimens of tibial marrow. Large changes are seen in the cellularity of the marrow during this phase, an initial drop from a mean of 136,000 per c.mm. on the first day to 35,000 on the ninth day, and a subsequent steady rise to 201,000 at three months. In view of these changes the total erythroid/myeloid and lymphoid counts per c.mm. provide more accurate indices of the different types of haemopoietic activity than do the percentages of these cells or the myeloid/erythroid ratio.

The marrow erythroid count at birth is about 41,000 per c.mm.; this falls to 2,800 at the ninth day and subsequently rises again to an average of 25,000 from two to three months: these changes are discussed further in Part II of this paper. The marrow myeloid count shows a similar fall at the end of the first week and a subsequent rise despite the constancy of the total number of myeloid cells in the circulation. The marrow lymphoid count shows a steady rise over the period studied, which parallels the rise in blood lymphocytes.

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