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LOCALIZED LEUKOCYTE MOBILIZATION STUDY IN THALASSEMIA, LEUKEMIAS AND SLE

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ABSTRACT :

Localized leukocyte mobilization (LLM) study using qualitative skin window technique originally described by Rebuck and Crowley are performed in 13 healthy Thai adults. It was found that the time relationship and percentage of different cell types migration into the local exudate are differed significantly from those described by Rebuck and Crowley but comparable to those observed more recently by many investigators. The normal pattern of LLM was also found in patients with thalassemia and acute myeloblastic leukemia in remission. Decreased macrophage migration into the local exudate was found in 3 patients with SLE.

INTRODUCTION

Localized leukocyte mobilization (LLM) is a complex phenomenon, involving changes in the local microcirculation, migration of leukocytes, permeability changes within the vascular endothelium, and the active process of leukocyte emigration out of the vessel. This sequence of events has been mainly studied in the rabbit ear chamber (1-3) and can be locally induced with a variety of mechanical, thermal, and chemical stimuli. The introduction of the skin window technique by Rebuck and Crowley (4) has greatly stimulated the qualitative study of induced inflammatory exudates in man. Several quantitative modifications of the coverslip technique have been attempted with varying results

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(5-7). Senn et al (7) by using the plastic skin-chamber technique have found three different patterns of leukocyte mobilization in normal individuals i.e. "peak", "up-slope" and "high-plateau" types. The physiologic significance of different kinetic leukocyte mobilization type is still unknown. In addition, even in the normal individual the number of leukocytes migrated into the skin chamber varied as much as 7 folds (7). In the light of the knowledge that the time relationships of leukocytic exudation into areas of experimental bacterial invasion is of great importance in limiting the spread of infection (8) and may somehow be involved in the initiation or stimulation of wound healing (9), the qualitative Rebuck skin window technique is adequate for clinical study.

Perillie in 1960 (10) confirmed earlier findings of Rebbuck and Crowley (4) that at about 1 to 1.5 hours following injury (skin window procedure) a heavy concentration of mature neutrophilic granulocytes was noted on the coverslip. This phase persisted for the first 6 to 8 hours of the nonspecific inflammatory cycle with only minor participation by histiocytes, lymphocytes and eosinophils. At the eighth to tenth hour, mature lymphocytes began appearing in increasing numbers and by the fourteenth hour they made up about 50 percent of the local cellular exudate. The next phase is the presence of sessible macrophages in an increasing number so that by the twenty-second to twenty-fourth hour they comprised over 80 percent of the cells present.

Kulapongs and associates (11, 12) in an attempt to study the defense mechanism in children with protein calorie malnutrition observed a unique pattern of LLM in healthy children and adults which is markedly different from those reported by Perillie and Finch (10). They have found that there is no significant number of lymphocytes present at any time and there is only moderate number of macrophages (less than 50 pecent) at the twenty fourth hour of the imflammatory cycle. These findings agreed with those of Senn et al (7) and more recently confirmed by Freyre and associates (13).

We are reporting our findings of the typical LLM pattern in normal healthy adults, the patients with acute leukemia, thalassemia and SLE.

MATERIAL AND METHOD

The normal subjects were 13 medical technology students. They were hematologically normal and free from illiness or recent infection. The method employed was a modification of the glass coverslip technique originally described by Rebuck and Crowley (4). The skin over a small

area of the volar surface was shaved, prepared with iodine and alcohol and then was scraped with a sterily scalpel until the capillary layer of the corium was exposed. The approximately 1 × 1 cm. lesion was then covered with a sterile coverslip which in turn was surmounted by a clean 1 × 1.5 inch glass slide and large adhesive tape. The cover slips were removed and replaced by a new one at 2, 4, 6, 8, 12 and 24 hours, air-dried, stained with Wright's stain and mounted on glass slides. The preparations were then examine microscopically. Each preparation was carefully examined with a low power lens and its relative cellularity determined and graded as 0 to 4+ (10) or scored by using grid (12) up to the maximum of 100. The differential cell count of 500 cells were done by the "fixed" method (14).

The stimulated lesion were done by applying a drop of tuberculin solution (1 : 1,000 dilution of old tuberculin) or diphtheria toxoid before applying the coverslip. Generally all 3 lesion sites of the "non-stimulated" and "stimulated" were done simultaneously on the same arm or the opposite arm.

RESULTS

The pattern of different cell types appeared in the local exudate on the coverslip is shown in Table I - III below. During the first 2 hours there are minimal cell migration to the skin lesion but most of them were polymorphonuclear neutrophils. The local exudates contained increasing amount of migrated cells with maximum between 16 - 24 hours. Results of of differential cell counts done in these healthy individual confirmed the previous findings or Kulapongs and Thongtong (11, 12) that the percentage of polymorphonuclear neutrophils (PMNs) in the local exudate decreases with time from almost 100 percent at 2 hour to about 50 percent at 24 hour. Eosinophils and basophils were present in a very small amount not exceeding 0.7 percent. Small lymphocytes started appearing in the local exudate at 4 hour with increasing amount to slightly over 1 percent at 24 hour. Monocytes and macrophages accumulated increasingly with time until reaching 45 percent range at 24 hour. The results obtained from stimulation with tuberculin aud diphtheria toxoid were practically identical to the nonstimulated lesion.

Normal pattern of LLM was found in 3 patients with beta thalassemia syndrome and 2 patients with AML during remission. Three patients with systemic lupus ery thematosus have shown an unusual pattern of decreased macrophage at 24 hour period even when stimulated with tuberculin or diphtheria toxoid. L.E. cell has never been found in the local exudate of these 3 patients.

TABLE I-III: TYPES OF CELLS MIGRATE TO THE SKIN WINDOW (MFAN±S.E.)

I. NON-STIMULATED PREPARATIONS

Hours	Neutrophil	Macrophage	Lymphocyte	Eosinophil	Basophil
2	99.69 ± 0.23	0.09 ± 0.08	0	0.22 ± 0.21	0
4	91.72 ± 1.86	7.53 ± 1.76	0.03 ± 0.03	0.66 ± 0.28	0.06 ± 0.04
6	76.87 ± 3.34	22.07 ± 3.21	0.37 ± 0.09	0.20 ± 0.09	0.08 ± 0.06
8	66.43 ± 4.49	32.85 ± 4.49	0.30 ± 0.13	0.28 ± 0.20	0.15 ± 0.09
12	58.02 ± 5.15	39.09 ± 5.29	0.81 ± 0.17	0.24 ± 0.10	0.28 ± 0.12
24	52.55 ± 4.24	44.53 ± 3.75	1.18 ± 0.27	0.23 ± 0.09	0.68 ± 0.27

II. STIMULATION WITH TUBERCULIN SOLUTION

Hours	Neutrophil	Macrophage	Lymphocyte	Eosinophil	Basophil
2	99.26 ± 0.27	0.28 ± 0.12	0	0.45 ± 0.21	0
4	93.40 ± 1 88	10.60 ± 1.93	0.08 ± 0.04	0.28 ± 0.13	0.06 ± 0.05
6	76.61 ± 2.97	21.93 ± 3.30	0.15 ± 0.67	0.49 ± 0.16	0.06 ± 0.05
8	62.51 ± 3.73	36.52 ± 3.67	0.30 ± 0.11	0.51 ± 0.15	0.15 ± 0.08
12	61.20 ± 5.33	36.00 ± 5.30	2.20 ± 0.19	0.40 ± 0.16	0.20 ± 0.19
24	55.90 ± 0.21	42.23 ± 6.73	1.28 ± 0.17	0.35 ± 0.13	0.60 ± 0.25

III. STIMULATION WITH DIPHTHERIA TOXOID

Hours	Neutrophil	Macrophage	Lymphocyte	Eosinophil	Basophil
2	98.71 ± 0.51	0.38 ± 0.16	0	0.52 ± 0.35	0
4	89.50 ± 2.07	14.12 ± 5.01	0.05 ± 0.03	0.68 ± 0.35	0.03 ± 0.03
6	72.05 ± 3.17	27.42 ± 3.16	0.11 ± 0.04	0.37 ± 0.14	0.05 ± 0
8	62.15 ± 3.17	35.62 ± 2.99	0.26 ± 0.11	0.49 ± 0.23	0.17 ± 0.07
12	56.18 ± 5.00	42.88 ± 5.25	0.66 ± 0.20	0.54 ± 0.26	0.32 ± 0.12
24	48.73 ± 0.95	49.09 ± 6.58	1.18 ± 0.29	0.35 ± 0.14	1.03 ± 0.38

COMMENTS

The morphologic characteristics of the inflammatory exudate are easily reproducible. The injury produced by the abrasion and the continued application of the coverslips is sufficient to reproduce the complete inflammatory cycle described by Rebuck and Crowley (4) using tuberculin solution and diphtheria toxoid. The neutrophilic granulocyte is considered the cell of major importance during earliest phase of the local inflammatory reaction which determines the success or failure of microbial invasion (15). Its prompt arrival in adequate numbers at the site of injury constitutes the first line of defense against such invasion. The delayed and diminished granulocyte response has been interpreted as a partial explanation for the susceptibility of leukemia (5, 10, 16, 17) uncontrolled diabetes (18), alcoholism (19), and a child with the presence of serum leukotaxis inhibitor (20), to infection. During early stages of inflammation, phagocytosis is attributable almost entirely to polymorphonuclear neutrophils (PMNs) probably for the simple reason that these cells are abundant in the circulation and most readily invade the area of injury. The PMNs are blood-borne as functionally mature cells and are attracted to the invading microorganisms or their product through positive chemotaxis. Bell et al (21), had demonstrated that the antibacterial functions of leukocytes derived from inflammatory exudates after dermal abrasion (skin window technique) and those derived from peripheral blood were comparable, suggested that, at least in normal subjects, leukocytes do not undergo a functional alteration during the complex process of tissue mobilization. Later in the inflammatory process, the major phagocytic cell type present is the macrophage. This cell type may either be blood-borne in the immature form, the monocyte (22-24), or may arise from the differentiation and proliferation of cells at the site of inflammation (25). Rebuck and Crowley's data (4) obtained with skin window technique supported the earlier believes that lymphocytes can transform to macrophage. Recent reports of both in vitro and in vivo experiment lend its strong support (24) In either case, maturation continues at the inflammatory site. Eosinophils and basophils are usually present in a very small percentages. Eosinophils are being postulated to represent a cellular aspect of the immediate-type hypersensitivity (26) since high eosinophil count is found in the skin window of atopic patient stimulated locally with offending allergen.

The typical LLM pattern in the patient with acute leukemia consisting of delayed in the initial PMN response. In comparison

with normal control, the initial response is sparse, and maximal cellularity develop more slowly (5, 10, 16, 17). These abnormalities returned to normal during remission as observed by these investigators and in our patients. The finding of decreased macrophage population in the local exudate of our patients with SLE is confirmed by the similar result obtained after diphtheria toxoid stimulation. These may related to the decreased cell-mediated immunity state as seen in malnourished children (12, 13). We have not seen L. E. cell in the local exudate as observed by Perillie et al (27). The association of frequent infections and thalassemia syndrome is well known. The underlying mechanism of its unusual susceptibility is still unkown. The review of this particular subject including its immunological status, phagocytic killing function, opsonin activity, Tuftsin activity and febrile response in thalassemia will be published by the author (P.K.).

CONCLUSION

Our findings of localized leukocyte mobilization study using qualitative Rebuck skin window in healthy Thai adults are significantly differed from those originally reported by Rebuck and Crowley (4) and Perillie et al (10) but identical to the more recent works by Senn et al (13). By using this technique, we have found that the LLM in the patients with acute myeloblastic leukemia in remission and patients with thalassemia are normal, but the macrophage migration or transformat on at the inflammatory site in patients with ESL are decreased significantly.

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ย่อเรื่อง

ได้ทำการศึกษาถึง Localized leukocyte Mobilization (LLM) คือการเคลื่อนที่ของเม็ดเลือดขาวมาบ้องกันเชื้อโรค ซึ่งจะเข้ามาทางบริเวณผิวหนัง โดยใช้วิธี qualitative skin window technique ของ Rebuck และ Crowley

ในการทดลองกับคนปกติ 13 คน พบว่าเม็ดเลือดขาวชนิดต่าง ๆ ที่ migrate ออกมาในช่วงเวลาหนึ่ง ๆ นั้น แตกต่างจากผลการทดลองของ Rebuck และ Crowley โดยสิ้นเชิง แต่ใกล้เคียงกับผลการทดลองอื่น ๆ ในระยะหลัง ๆ อีกหลายอัน

นอกจากนี้ยังได้พบอีกว่า คนไข้ที่เป็น Thalassemia และ Myeloblastic Leukemia ก็ได้ผลแบบเดียวกับคนปกติทุกอย่าง แต่คนไข้ 3 คนที่เป็น SLE จะมี macrophage ออกมาน้อยลงกว่าปกติ.