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BASOPHIL LEUCOCYTE RESPONSE IN LEPROSY WITH LEPROMIN - A

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Study of 'Basophil Leucocyte Response' in two polar groups of leprosy patients (tuberculoid and lepromatous) was undertaken employing Lepromin-A (Armadillo) by the method of Open Window Technique of Rebuck. Significant infiltration of Basophils at the test side is demonstrated both at 48 hours and at 72 hours in lepromatous leprosy as compared to the control groups i.e., in placebo and tuberculin positive healthy volunteers as well as against tuberculoid leprosy group (p value being less than 0.05 and 0.001). The basophils also go into degeneration and degranulation at 72 hours.

The role of basophils in the different types of allergic reactions is discussed in context of above finding. The possible effect of high infiltration of basophils in lepromatous leprosy with its subsequent release of histamine and other factors by degranulation, and its possible role in immuno-regulation through specialised suppressor cells is postulated in the light of available data.

Introduction

Leprosy is one of those diseases where inspite of extensive studies, the precise nature of the immune defect is still not yet fully understood, especially nature of specific depression of cell- mediated immunity in lepromatous leprosy, as evidenced by negative delayed hypersensitivity reaction.

Although T-lymphocytes and monocyte-macrophage system are the principal essential participants in delayed hypersensitivity skin reaction, it has been noted that products of B-cells, eosinophils, mast cells/basophils may also be involved in varying degrees.

During last one and half decade interest has been created on the role of basophils in delayed hypersensitivity reaction as a result of the work of Dvorak and his colleagues[1,2] who demonstrated their participation in such reaction by degranulation and release of histamine. Basophils are found immediately under the epidermis in most delayed hypersensitivity reactions especially in allergic contact dermatitis and Jones - Mote reactions.[3] Fulton and Derbes[4] also demonstrated the presence of basophils in variable amount in dermal exudate in positive tuberculin skin test reaction. In order to determine, whether similar processes might be involved in the delayed hypersensitivity reaction in leprosy with Lepromin - A (Lepromin prepared from tissue of Armadillo infected with M. Leprae, containing 40 million dead bacilli per ml of the emulsion) the present study has been undertaken using the skin-window technic developed by Rebuck (Fulton and Derbes).[4]

Materials and Methods

Thirty seven patients of leprosy (twenty tuberculoid and seventeen lepromatous) age - group between 12 to 40, eleven females and rest males, were selected for this study. Only patients of

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two polar types i.e., tuberculoid and lepromatous based on the criteria laid down by Indian classification were chosen. Eleven tuberculin positive volunteers between the age group of 16 - 30 years with no evidence of active tuberculosis served as controls.

The following protocol was worked out. On the first day, either 5 T.U. of Old tuberculin, or 0.2 ml of Lepromin-A, or 0.2 ml of Phenol-saline (0.5% phenol in normal saline) was injected intradermelly in the middle third of the volar surface of the forearm using 0.1 ml tuberculin syringe and a 26 gauge hypodermic needle.

Twenty-four hours later, on second day a 15 mm diameter (approx.) area of epidermis of the resulting reaction was scraped off with sterile scalpel blade. An area of about 15 mm diameter was scraped down to the rate ridges. Care was taken that the lesions did not bleed. Following the removal of the skin, sterile round glass cover-slips (18 mm size) were placed over the lesions. Sterile gauge pads were put over the glass to keep the cover-slips from breaking. Finally the material was taped in place with leucoplast and the whole area was bandaged.

Four hours later i.e., at 28 hours after the initial injection, the first cover-slip was removed and another was put in its place. A similar exchange was done at 48 hours and at 72 hours.

The cover-slips were stained with Leishman's stain (G.T. Gurr, London) and mounted on a microscope slide with DPX. On each slide two hundred cells were counted in the area that yielded the highest aggregation of cells. The cells were classified in four groups:

(i) Neutrophilic granulocytes.

(ii) Mononuclear cells (lymphocytes, monocytes, macrophages)

(iii) Eosinophilic granulocytes.

(iv) Basophilic granulocytes.

Results

The findings have been depicted in the micro-photographs and tables. Fig. 1 shows that at 28 hours in leprosy (both tuberculoid or lepromatous) there is an exudate consisting of 80-90 percent of neutrophilic granulocytes. It is also true for tuberculin positive group as demonstrated by Fulton & Derbes[4] and same is the case of phenol-saline group (Table I).

At 48 hours the character of exudate is changed (Fig. 2). No longer the neutrophils are the predominant cells. Mononuclear cells have appeared in large number, as well as a few eosinophils. But the most significant change is the influx of basophils and the differential count of cell exudate shows (Table II) that the influx is more in lepromatous leprosy than that in tuberculoid leprosy or in tuberculin positive group. Even in tuberculoid leprosy there appears to be more basophils than that in tuberculin positive group. In phenol-saline group, however there is no influx of basophil at all .

At 72 hours, the basophils have become hypertrophic and distorted. Most of the basophils have degranulated. Quite a few mononuclear cells have taken up the granules (Figs. 3,4). Again the differential count shows (Table III) that the influx of basophils is highest in lepromatous group and gradually decreasing in tuberculoid and tuberculine positive group and almost

absent in phenol-saline group, as has been observed at 48 hours.

In Table IV the influx of basophils on lepromatous group has been compared stastically to other groups employing Student's t-test. The influx does not appear to be significant stastistically at 28 hours with any of the other groups p value being more than 0.05.

At 48 hours and at 72 hoyrs however the influx of basophils in lepromatous group appears to be highly significant as compared to phenol-saline group or tuberculin positive group or even tuberculoid leprosy group, the p value being less than 0.001 in most of the cases.

Comments

Basophils leucocytes (and/or mast cells) exhibit chemotaxis and phagocytic activity but their physiologic role is played by discharge of the contents of the specific granules to the exterior.[5] Probably, the human basophils contain all the blood histamine localised in its granules. Other than histamine, the granules also contain heparin, 5-hydroxytryptamine, eosinophil and neutrophil chemotactic factors, platelet activating factors besides other factors having known and unknown physiologic role.

Basically the basophils (and/or mast cells) are well-established for their role in IgE mediated Type I hypersensitivity

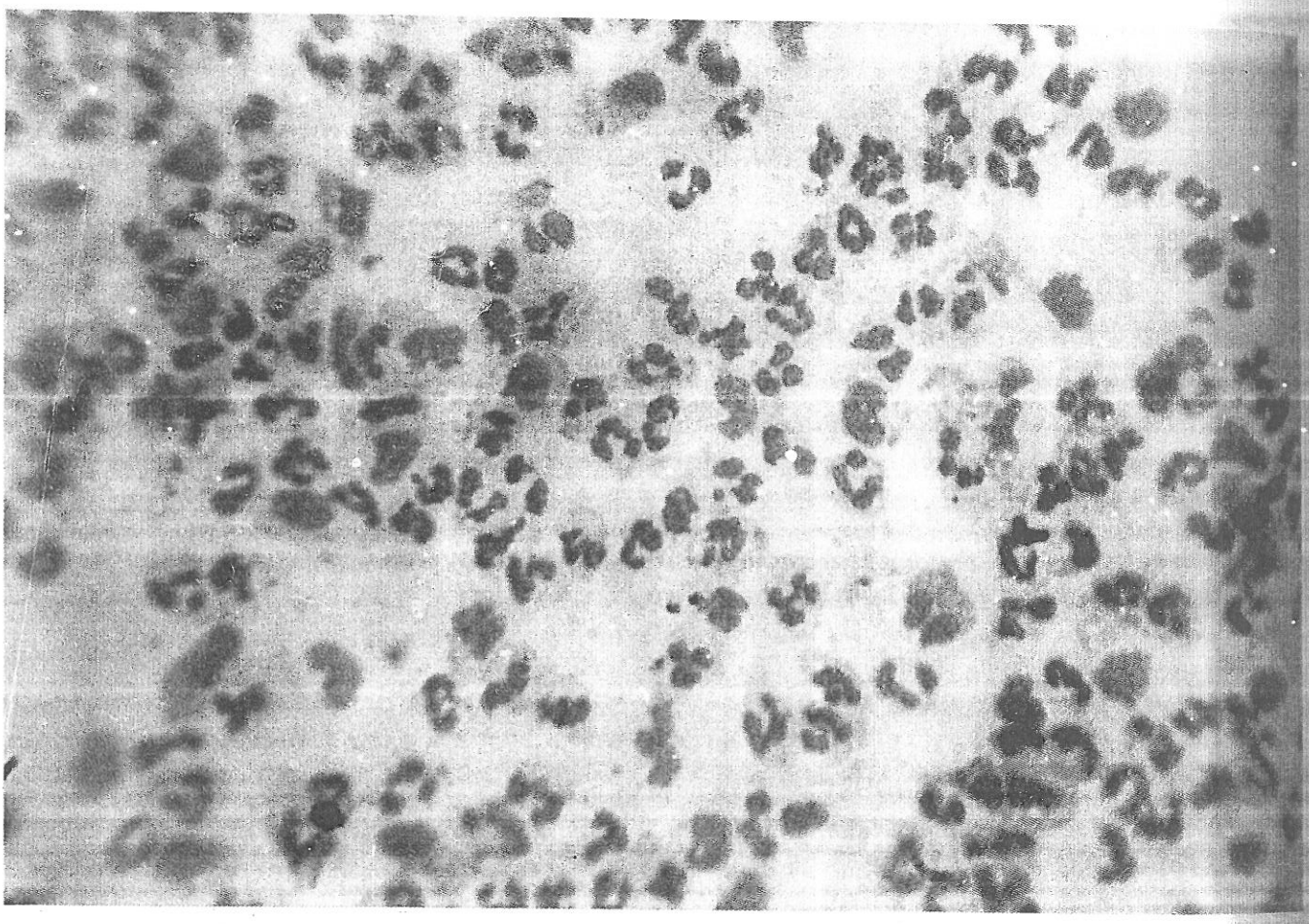

Fig. 1. Microphotograph showing cellular exudate in lepromatous leprosy at 28 hours (low magnification x 10)

reaction. The interaction between the antigen and the IgE attached to the surface of basophils initiate a sequence of reactions furthering imbalance of cyclic neucleotides (i.e., cyclic AMP and cyclic GMP) which leads to degranulation and release of histamine and other substances.

Although the relevance of IgE and basophils/mast cells to Type I type of hypersensitivity reaction is well known, it must not be forgotten that a wide variety of agents may act to degranulate human basophils like radio-contrast media, opiates, curare, dextran, irradiation etc.[6] Rather than diminish the role of basophils/mast cell in immunologic diseases, this fact, instead, expands the relevance of this cell and its many mediators to other phathophysiologic states independent of antigen or antibody.

Several studies have shown increased histamine level at the sites of contact allergic reactions.[7,8] Basophils in the blister fluid and the skin window exudate of contact allergic reaction sites have been well documented.[9,10] Dvorak et al[1,2] think that their presence can be explained by the co-existence of delayed hypersensitivity reaction of Jone's Mote Type together with conventional "Lymphocyte- macrophage mediated" delayed hypersensitivity.

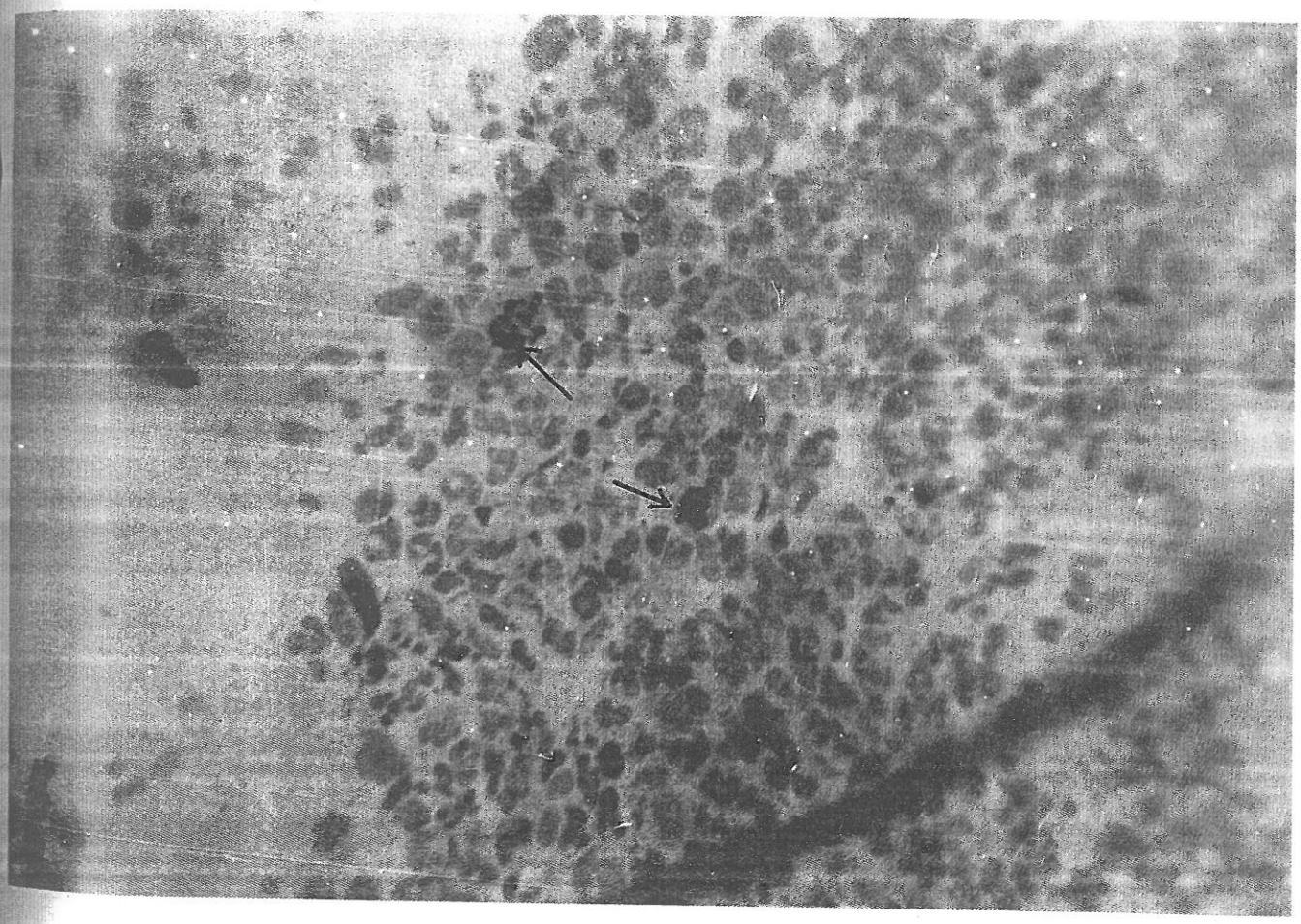

Fig. 2. Microphotograph showing cellular exudate in lepromatous leprosy at 48 hours (low magnification x 10)

Now this "Jone's Mote Reaction" or so-called "cutaneous basophil hypersensitivity" is another interesting aspect of immune reaction. These transitary reactions induced by antigen with or without Freund's incomplete adjuvant (as against antigen with Freund's complete adjuvant) have been shown to be the result of more intense immuno-regulation by specifically induced supressor cells by Turk et al[11] and Ota et al[12] in a series of studies using cyclophosphamide to elimitate suppresor cell precursors.

How this regulatory mechanism works in the periphery is still not clearly understood, but it has been proposed by Askenase[13] that B- cell produce IgG antibody locally which acts through the basophils which then produce vaso-active amines locally and dampen the delayed hypersensitivity reaction. Such type of immuno-regulatory process by suppressor cells has been demonstrated in contact sensitivity to simple chemical sensitizer like DNCB and picryl chloride by many workers including Chase[14] and Turk et al.[15] Askenase et al[16] have further elucidated the role of these suppressor cells which they name "histamine dependent suppressor cells", in enhancing melanoma tumor growth in a series of experiments using animal model.

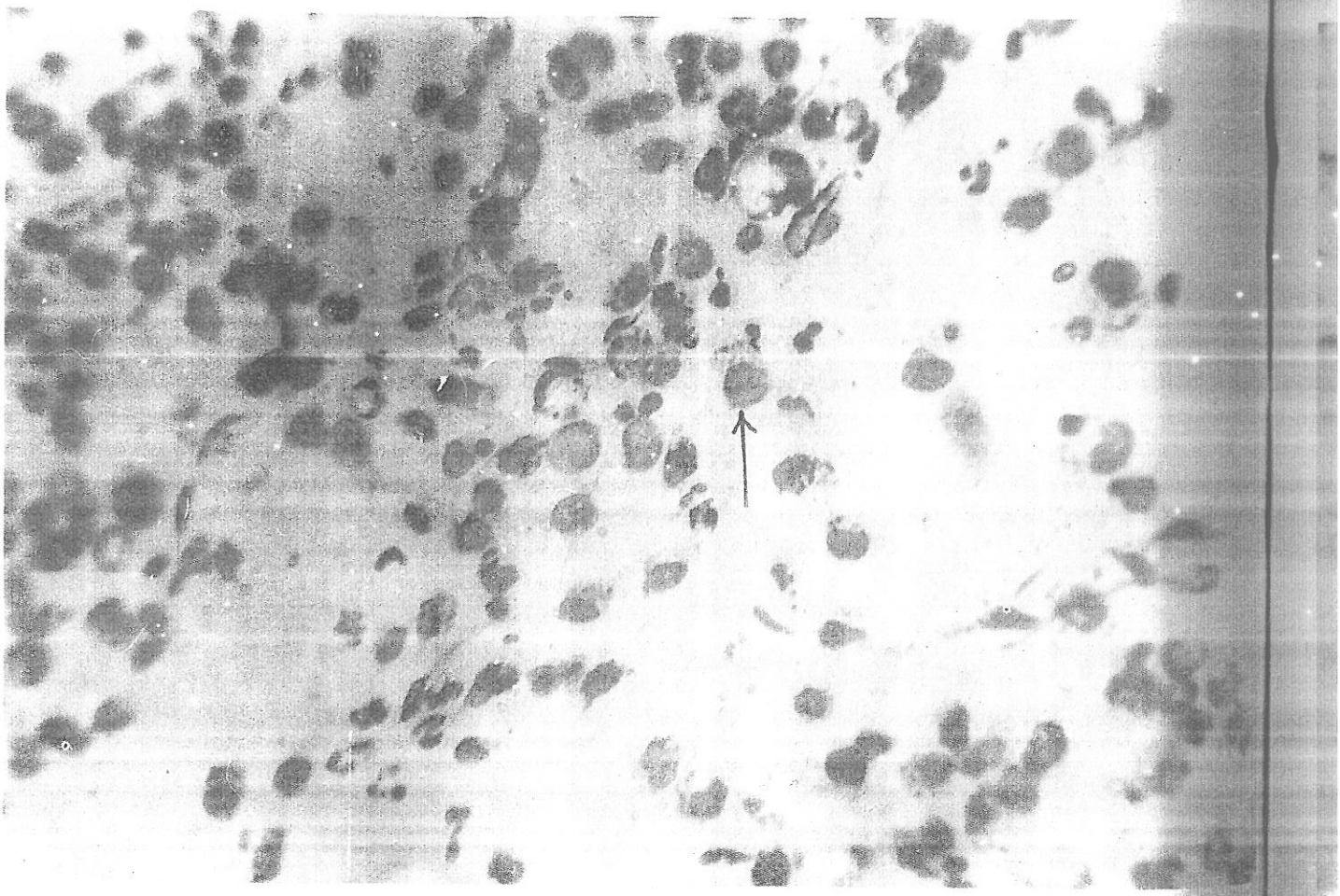

Fig. 3. Microphotograph showing cellular exudate in lepromatous leprosy at 72 hours (low magnification x 10)

What would be the role of basophils in the delayed sensitivity reaction in leprosy with Lepromin - A is more a matter of speculation. Increase in number of mast cells have also been observed in human and murine leprosy.[19] Kumar et al[20] demonstrated increased level of serotonin in the blood of lepromatous patient, significance of which remains to be determined. In a comprehensive review on the subject of possible immune-depression in leprosy, Turk & Curtis[15] feel that the failure could lie at any level starting from monocyte-macrophage system to at T-cell level. They have also laid stress on the role by suppressor cells of specific immuno-regulation, a fact emphasised by Mehra et al.[21] Whether these specialized suppressor cells are "histamine dependent" as envisaged by Nordlund and Askenase[16] or bear H_2 type histamine receptors (Shearer et al[18] as quoted by Nath[17]) or induced by preferential stimulation to large mycobacterial antigen as postulated by Turk et al[15] shall remain a matter of future investigation. And thus the present study demonstrating significant basophil infiltration in lepromatous leprosy as compared to tuberculoid leprosy or controls might throw some light to this particular branch of research.

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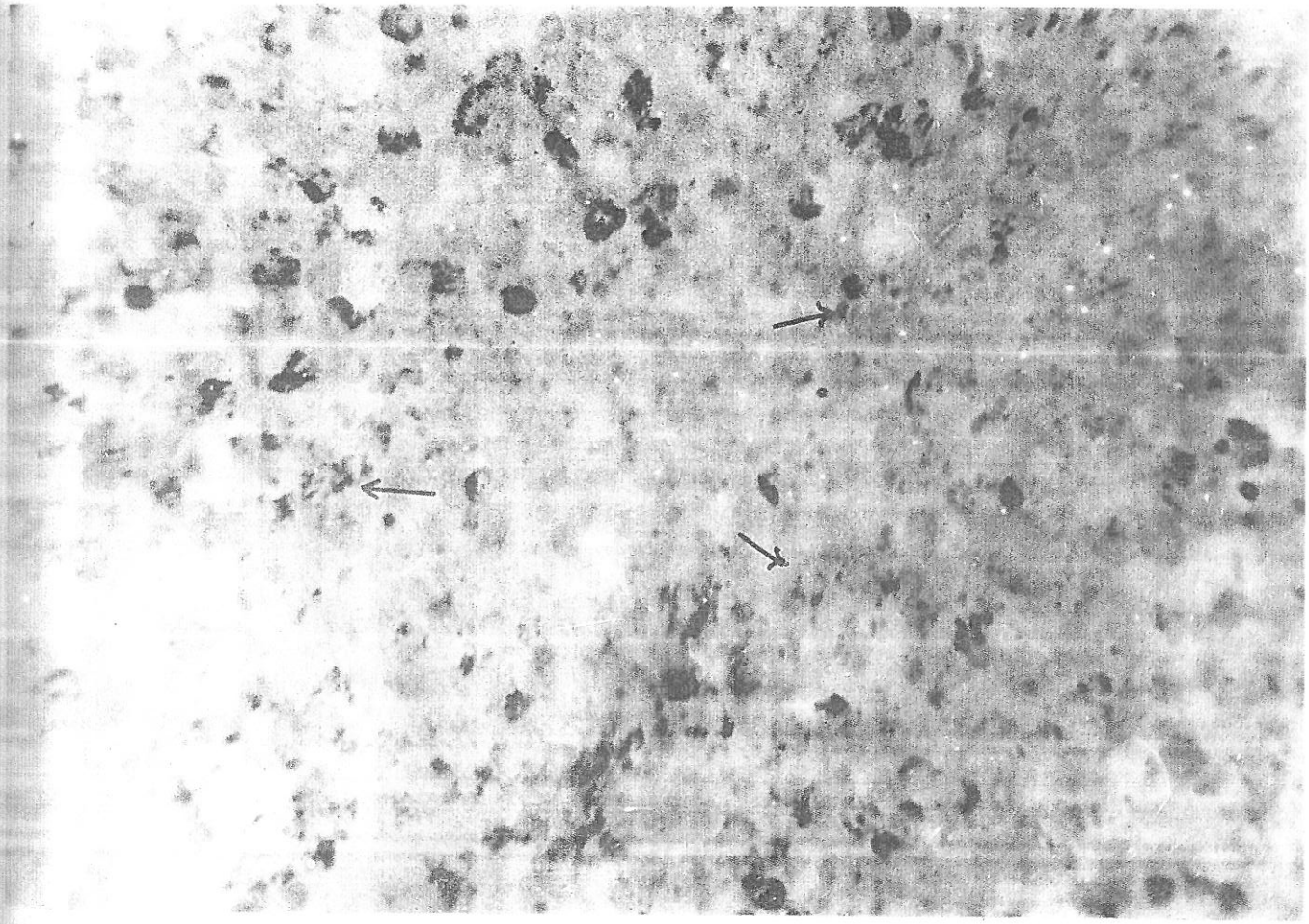

Fig. 4. Same as Fig. 3 at higher magnification (x40)

Table I. Exudate cell - picture at 28 hours (200 cells)

Cells	Phenol saline group (5 people)	Tuberculin - positive group (6 people)	Leprosy group	
			Tuberculoid (20 patients)	Lepromatous (17 patients)
Neutrophils	182.4 SD ± 5.13	172.6 SD ± 14.67	169.8 SD ± 26.2	185.53 SD ± 6.44
Mononuclears	10.3 SD ± 2.39	20.5 SD ± 10.93	25.9 SD ± 24.77	12.47 SD ± 6.13
Eosinophils	6.8 SD ± 2.95	6.2 SD ± 5.91	3.25 SD ± 2.38	1.29 SD ± 0.39
Basophils	0.0 SD ± 0.0	0.7 SD ± 0.31	1.1 SD ± 1.14	0.63 SD ± 0.77

Table II. Exudate cell - picture at 48 hours (200 cells)

Cells	Phenol saline group (5 people)	Tuberculin - positive group (6 people)	Leprosy group	
			Tuberculoid (20 patients)	Lepromatous (17 patients)
Neutrophils	105.4 SD ± 8.3	113.5 SD ± 44.59	81.15 SD ± 18.97	98.7 SD ± 19.72
Mononuclears	92.4 SD ± 9.1	72.0 SD ± 39.21	108.45 SD ± 19.49	83.7 SD ± 17.73
Eosinophils	1.2 SD ± 0.5	3.3 SD ± 6.86	0.5 SD ± 0.5	0.62 SD ± 0.49
Basophils	1.0 SD ± 0.33	10.7 SD ± 6.18	10.85 SD ± 3.41	17.52 SD ± 6.58

Table III. Exudate cell - picture at 72 hours (200 cells)

Cells	Phenol saline group (5 people)	Tuberculin - positive group (6 people)	Leprosy group	
			Tuberculoid (20 patients)	Lepromatous (17 patients)
Neutrophils	44.0 SD ± 5.1	38.2 SD ± 18.29	50.2 SD ± 18.7	49.93 SD ± 13.03
Mononuclears	151.6 SD ± 4.94	144.7 SD ± 14.21	130.7 SD ± 21.03	118.0 SD ± 10.74
Eosinophils	2.2 SD ± 0.84	1.3 SD ± 1.25	0.35 SD ± 0.48	0.35 SD ± 0.48
Basophils	2.2 SD ± 0.83	15.8 SD ± 6.41	20.95 SD ± 4.63	32.52 SD ± 7.63

References

1. Dvorak HF, Dvorak AM, Simpson BA, Richerson HB, Leskowitz S, Karnovsky MJ. Cutaneous basophil hypersensitivity. II. A light and electron microscopic description. J Exp Med 1970; 132 : 556-82.

Table IV. Stastistical consideration of basophil leucocyte response (Student's t-test)

Time	Comparision of lepromatous group with other groups	P. Value	D.F.	Significance level
28 hours	I. Compared to Ph.Saline group	> 0.05	20	Not significant
	II. Compared to tuberculin positive group	> 0.05	21	Not significant
	III. Compared to tuberculoid leprosy group	> 0.05	35	Not significant
48 hours	I. Compared to Ph.Saline group	< 0.001	20	Highly significant
	II. Compared to tuberculin positive group	< 0.05	21	Significant
	III. Compared to tuberculoid leprosy group	< 0.001	35	Highly significant
72 hours	I. Compared to Ph.Saline group	< 0.001	20	Highly significant
	II. Compared to tuberculin positive group	< 0.001	21	Highly significant
	III. Compared to tuberculoid leprosy group	< 0.001	35	Highly significant

2. Dvorak HF, Mihm NC Jr, Dvorak A. Morphology of delayed-type hypersensitivity in man. J Invest Dermatol 1976; 67 : 391-401.
3. Fregert S, Rorsman H. Basophil leucocyte in photo-contact dermatitis. J Invest Dermatol 1964; 42 : 405-6.
4. Fulton JE, Derbes VJ. Basophil leucocyte infiltration in the positive tuberculine skin test in man. J Invest Dermatol 1964; 43 : 125-8.
5. Maiale JB. Basophils leucocytes. In: Laboratory Medicine : Hematology. St. Louis : C. V. Mosby 1982; 665.
6. Wasserman SJ. The mast cell and its mediators. In : Biochemistry and Physiology of the skin (Goldsmith LA, ed). USA: Oxford University Press, 1983; 878-98.
7. Zachariae H. Histamine in delayed skin reactions. Fluorometric determinations on patch tests. J Invest Dermatol 1964; 42 : 431-4.
8. Aoki K, Saito T. Contact dermatitis and histamine. Jap J Dermat Ser B 1972; 82 : 33-40.
9. Baer RL, Yanowitz M. Differential cell counts in the bilister fluid of allergic eczematous and irritant bullous reaction. J Allergy 1952 : 23 : 95-103.
10. Wolf-Jurgensen P. Basophil leucocytes in delayed hypersensitivity. Experimental studies in man using the Skin Window Technique. Munksgaard, Copenhagen, 1966.
11. Turk JL, Polak L, Parker D. Control mechanisms in delayed-type hypersensitivity. Br Med Bull 1976; 32 : 165-70.
12. Ota F, Parker D, Turk JL. Further evidence for non T-cell regulation of delayed hypersensitivity in the guinea pig. Cell Immanol 1976; 43 : 263-70.
13. Askenase P W. Roleof basophils, mast cells and vasoamines in hypersensitivity reactions with a delayed time course. Progr Allergy Vol 23. Basel : S Karger, 1977, 199-320.
14. Chaso MW. Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. Proc Soc Exp Biol Med N Y 1964, 61: 257-9.
15. Turk JL, Curtis J. Possible mechanism of immunodepression in Leprosy. In : progress in Immunology of Leprosy (Talwar GP, Turk JL, Rees RJW, eds). New Delhi : Arnold-Heinemann Publishers (India), 1983, 87-99.
16. Nordland JJ, Askenase PW. The effect of histamine, antihistamines and a mast cell stabilizer on the growth of Cloudman melanoma cells in DB A/2 mice. J Invest Dermatol 1983; 81 : 23-31.
17. Nath I. Immunological aspects of human leprosy. Lepr India 1983; 55 : 752-62.
18. Shearer GM, Melmon KL, Weinstein Y, Sela M. Regulation of antibody response by cells expressing histamine receptor. J Exp Med 1972; 136 : 1302.
19. Kato L, Gozzy B. Mast cell response in murine leprosy. Int J Lepr 1965; 33 : 50-60.
20. Kumar R, Vaidya MC, Belurker N. Blood levels of Serotonin in human Leprosy. Lepr India 1980; 52 : 532-5.
21. Mehra V, Convit J, Rubinsteen A, Bloom BR. Activated Supressor T cells in Leprosy. J Immunol 1982; 129 : 1946-51.