Acta Dermatovener (Stockholm) 51: 135–137, 1971

DIMINISHED PHAGOCYTIC FUNCTION IN PATIENTS WITH ALOPECIA

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Abstract. Diminished ability of the circulating granulocytes to phagocytize yeast cells (*Saccharomyces cerevisiae*) in vitro was found in a group of 21 apparently otherwise healthy patients with unexplained hair loss. This finding was present in patients with accelerated hereditary hair loss as well as in those with alopecia areata and other unexplained alopecias. Crossover studies with normal plasma and normal white cells did not permit identification of a single cellular or humoral locus for the defect. The degree of depression of phagocytic function could not be correlated with the extent, duration or severity of hair loss. Although a phagocytic defect characterized this group, the nature of its relationship to hair loss is obscure.

Many diseases regularly cause alopecia. Nonetheless, they do not account for alopecia areata, nor for the accelerated hair loss experienced by an increasing number of individuals seeking medical help (2). Muller & Winkelmann in a comprehensive review of 736 patients with alopecia areata failed to find a consistently significant role for the following conditions classically associated with alopecia areata: atopic dermatitis, neuropsychiatric problems, acute emotional stress, headaches, head injury, thyroid disease and diabetes mellitus (4). Many observers have also written on the nature of chronic diffuse hair loss. Sulzberger et al. have reviewed this problem in detail, but adduced no evidence that any of the following factors was causative: topically applied formulations, endocrine state, psychosomatic status, trauma, seborrheic dermatitis, medications, operative procedures, food sensitivities, environment, or simply increased awareness on the part of the patient (7). Most certainly the pathogenesis of accelerated hair loss in the presence of normal skin and in the absence of systemic disease is unknown.

In the course of our study of this problem, we observed that many patients with diffuse idiopathic loss of hair demonstrated a blue fluorescent spot on urinary chromatography (5). This spot proved to be para-amino hippuric acid which in turn reflected the unusually frequent intake of aspirin by these individuals. The present study arose as a result of a similarly intriguing observation made in our laboratory; namely, a young boy with alopecia areata was observed to have a striking reduction in the capacity of his white cells to ingest yeast cells.

METHODS

The phagocytic capacity of polymorphonuclear leucocytes was studied by a modification of the technique of Brandt (1) in which 10 ml of blood was drawn into a heparinized syringe, mixed by gentle inversion, and allowed to sediment for 1 hour. The buffy coat was then removed, washed twice with heparinized saline solution and reconstituted to a concentration of five million leucocytes per ml. Yeast cells (*Saccharomyces cerevisiae*) were diluted to a concentration of one billion per ml. Tubes prepared for incubation contained 0.1 ml of 40% plasma in Earle solution. The yeast to leucocyte ratio was maintained at 100 : 1 throughout the test. In each instance the system was tested four ways: with patients' cells and patients' plasma, with patients' cells and normal plasma, with normal cells and normal plasma, and with normal cells and patients' plasma. To increase random contact of yeast particles with leucocytes, the tubes were rotated for 30 min at 37°C. After the tubes were then centrifuged at 500 rpm for 4 min, the sediment was smeared, air-dried and stained with Wright stain. The number of yeast cells phagocytized by 100 leucocytes was counted and the phagocytic index was calculated as the average number of yeast phagocytized per leucocyte.

Because there is an unavoidable variation in the yeast preparations as well as in the phagocytic capacity of the normal subjects who served as daily controls, the results are reported as a percentage of the highest phagocytic

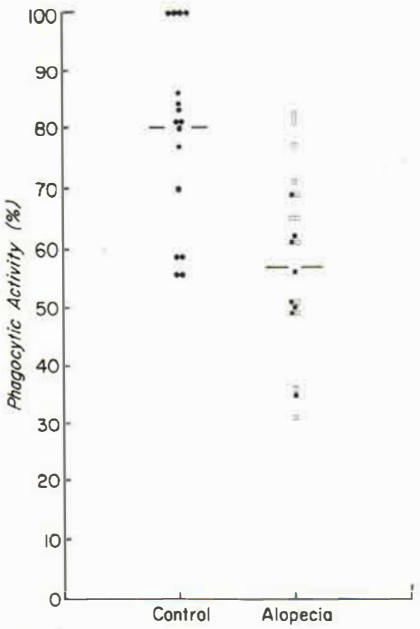

Fig. 1. The phagocytic activity of granulocytes from a control group of 16 dermatologic patients without hair loss and from the 21 patients with alopecia. ■, Alopecia areata; □, accelerated alopecia.

index of each series of determinations—i.e. if in a day's run the highest phagocytic index was 4.0, a single test with a result of 3.0 would be reported as 75%. Reproducibility of the technique was tested by repeating the series of determinations on 6 patients two to six times over a period of several days to weeks. Analysis of these results gave a value of ±12% for one standard deviation.

PATIENTS

For the test group the 21 patients were not randomly selected from the general population of bald or balding people, but represented a group of individuals who were experiencing sufficiently accelerated hair loss to seek dermatological consultation. This group was heterogeneous in that it included patients with alopecia areata, alopecia totalis, as well as patients with accelerated alopecia (chronic diffuse alopecia of unknown origin). Excluded from this series were patients with skin disease of the scalp, patients with significant systemic disease, and finally patients with patterned hair loss. Used as a control group were 16 patients with dermatologic diseases without any complaint or evidence of accelerated hair loss.

RESULTS

The group of patients with hair loss had a mean value for phagocytic activity of 58% (S.D. 14.3%) which was significantly lower ($P < 0.001$) than the average of 80% (S.D. 15.9%) found in the control group (Fig. 1). The values found in this control group were the same as those reported by Miller et al. (3) Using the same method for evaluating phagocytosis, they found that incubation of normal leucocytes with the plasma from 100 adults gave a mean value of phagocytic activity of 84.9% (S.D. 11.3%). Replacing the patient's cells or plasma with normal cells or plasma had a variable effect in the tests on individual patients in the hair loss group but, on the average, did result in increased phagocytic activity. The average increase was similar with the addition of either normal cells or normal plasma and was statistically significant ($P < 0.05$). In the control group no such enhancement was found.

DISCUSSION

The probe observation that the granulocytes of a young patient with alopecia areata had a strikingly reduced capacity for phagocytosis stimulated this study of a group of patients with hair loss. This finding was extended and proved to be singular in that previously extensive laboratory studies on 10 patients with alopecia areata, hospitalized in the Clinical Research Center, had shown no abnormalities in examinations as disparate as: fat balance studies, intestinal biopsies, skin inflammatory cycles, hematological workup, endocrine analyses, liver function tests, as well as a battery of standard laboratory procedures (6).

Not only did the hair loss patients as a group have a statistically significant lower mean phagocytic activity but not one of the 21 had a phagocytic index equal to or exceeding his normal healthy individual control mate. In contrast, 4 of the 16 in the control group of dermatologic patients did show a phagocytic activity greater than his paired normal healthy control mate. Although the subjects with alopecia areata tended to have lower values for the phagocytic indices the difference from the other patients with hair loss was not significant. It was not possible to relate the degree of the defect in phagocytosis with the duration, severity or extent of hair loss. Neither steroid therapy (3 patients) nor spontaneous regrowth of hair (2 patients) was associated with any change in the phagocytic activity. Since addition of either normal plasma or normal cells to

the specimens from the patients resulted in a significant improvement in the phagocytic index, the defect cannot be identified as totally cellular or humoral.

The data are restricted to obsevrations on the phagocytosis of *S. cerevisiae* and give no specific information on the granulocytes' ability to handle bacteria or other microflora. In this regard a special attempt was made to assess the ability of the cells to ingest *Pityrosporum ovale*, a related yeast and common inhabitant of the scalp. This was unsuccessful because the *P. ovale* could not be grown so that the organisms did not agglutinate.

The significance of the reduced phagocytic function in these patients with alopecia is not clear. The ability to phagocytize and to produce proteins are two functions common to many cells. In the granulocyte, phagocytosis is highly developed whereas in the hair follicular cells, the production of the protein, keratin, is the specialized activity. Since the loss of hair without replacement signifies a defect in the specialized function of the rapidly turning over follicular cells of the hair bulb and since diminished phagocytosis signifies a defect in the granulocytes, both of these changes may be a sensitive indicator of a toxic or metabolic effect. However, at this time there is no direct evidence to explain the present observation of a diminished phagocytosis and its relationship to alopecia.

ACKNOWLEDGEMENTS

This work was supported by a grant from the John A Hartford Foundation and by Clinical Research Center grant 5MO1-RR-40, Division of Research Resources, National Institutes of Health.

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Received September 11, 1970

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