
Monocyte Function in Idiopathic Nephrotic Syndrome in Childhood

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Department of Pediatrics, Hirosaki University School of Medicine, Hirosaki 036, *Iwaki Hospital, National Sanatorium, Pediatrics, Namioka 038-13, and †Hirosaki National Hospital, Pediatrics, Hirosaki 036

WAGA, S., TAKAHASHI, Y., FUJITA, M., NAGATA, K., KURONUMA, T. and AYOYAMA, R. Monocyte Function in Idiopathic Nephrotic Syndrome in Childhood. Tohoku J. exp. Med., 1983, 141 (3), 295-303 — The monocyte function of 112 specimens from 42 children with idiopathic nephrotic syndrome (INS) aged from 2 to 17 years was studied by the methods of nitroblue tetrazolium (NBT) reduction, phagocytosis of immunobeads (IB) and yeast cells, chemotaxis and acid alphalphanaphthyl acetate esterase (ANAE) staining. The dissociation between phagocytosis and chemotaxis was observed in the fresh cases of steroid sensitive INS and in the cases of steroid non-sensitive INS. In the fresh cases of steroid sensitive INS, NBT reduction and phagocytosis were increased, but chemotaxis was decreased. In the cases of steroid non-sensitive INS, the phagocytosis of IB was decreased, but chemotaxis was increased. These findings suggest a different pathogenesis between steroid sensitive and steroid non-sensitive INS. The dissociation between phagocytosis and chemotaxis may be explained by the alteration of the surface receptors of monocyte and by lymphokines.

Several observations, including those on abnormality of T cell function (Wissermann et al. 1977; Nagata et al. 1979; Kuronuma 1981), suggest an immunopathogenetic mechanism for idiopathic nephrotic syndrome (INS) (Glassock 1979). There have been a few studies of the monocyte function in INS, while it is known that the monocyte-macrophage series plays a central role in the induction of the immune response with respect to the presentation of antigen to T cells, and that the function of macrophage changes in several diseases (Ralph and Mergenhagen 1976). In our previous paper we reported the monocyte function in INS children in reference to the phagocytosis and nitroblue tetrazolium (NBT) reduction (Nagata et al. 1981). In this paper, we describe the monocyte function in

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INS children investigated by the methods of NBT reduction, phagocytosis, chemotaxis, and acid alpha-naphthyl acetate esterase (ANAE) staining.

**MATERIALS AND METHODS**

*Subjects.* Fifty-eight cases of INS children aged from 2 to 17 years were divided into two groups; the one (45 cases, 84 specimens) which responded to steroid treatment and the other (13 cases, 28 specimens) which did not respond. The former group was further subdivided into four subgroups; 10 fresh cases which had not been treated with steroid, 9 cases (11 specimens) of relapse, 17 cases (41 specimens) of incomplete remission, and 9 cases (22 specimens) of complete remission. Histologically, this group showed minimal abnormalities. The latter group included membranoproliferative glomerulonephritis (MPGN) (9 cases, 22 specimens), refractory nephrotic syndrome (3 cases, 3 specimens), and chronic glomerulonephritis (1 case, 3 specimens).

The preparation of mononuclear cells (MNC) from peripheral blood. MNC were prepared by centrifugation of 5 ml of heparinized peripheral blood on a Ficoll metrizoate gradient and washing the cells twice with 0.15 M phosphate buffered saline solution (PBS, pH 7.2). The purity of MNC was almost 100% and viability of these cells was over 96%.

NBT reduction of monocytes. This was examined by the method of Park et al. (1968). Details have been described in our previous report (Nagata et al. 1981).

Phagocytosis of immunobeads (IB). 1B were adjusted at a concentration of 2.5 X 10^7/ml. Suspension of MNC and IB were mixed in equal amounts and the mixture was allowed to stand at 37°C for 60 min in a water bath. One drop of the mixture was taken on slide glass, which was allowed to place at 37°C for 60 min, and was washed away by Hanks balanced salt solution (BSS). After the fixation of adherent monocytes with methanol and Giemsa staining, the percentage of IB phagocytosing monocytes was calculated.

Phagocytosis of yeast cells. Baker's yeast cells, previously treated by boiling at 100°C for 30 min, were adjusted at a concentration of 2 X 10^8/ml. Suspension of MNC and yeast cells were mixed in equal amounts. Then the mixture was allowed to stand at 37°C for 60 min. After one drop of this mixture was taken on slide glass, phagocytosing monocytes were calculated in the same procedure as that for IB phagocytosis.

Chemotactic reaction of monocytes. The agarose plate method of Nelson et al. (1975) was used. One ml of fresh serum of a healthy adult containing 25 mg of Zymosan (Sigma Co.) was placed at 37°C for 30 min, and was centrifuged at 3,000 rpm for 5 min. The supernatant was used as a chemotactic factor after being allowed to stand at 56°C for 30 min. Five ml of 1.2% agarose (Hoechst Co.) resolved in RPMI 1604 (GIBCO Co.) containing 15% fetal calf serum (FCS) were poured in a Petri dish (Coster Co. 60 X 15 mm). Three wells were punched out in agar gel straight 3 mm in diameter and at intervals of 5 mm. In the central well, 10 μl of MNC were poured, and in the other two wells 10 μl of chemotactic factor and 10 μl of RPMI 1640 (control solution) were poured separately. After placing at 37°C for 20 hr in 5% CO₂, this preparation was fixed in formalin for 10 min. Then the agarose was taken off from the Petri dish and was stained by the Wright method. The distance between migrated monocyte and well containing Zymosan was assessed as chemotaxis, and that between migrated monocyte and well containing RPMI 1640 was estimated as random migration. The chemotactic index was evaluated as the ratio of chemotaxis to random migration (chemotactic index = chemotaxis/random migration).

Acid alpha-naphthyl acetate esterase (ANAE) staining of monocytes. MNC were suspended in 4 ml of RPMI 1640 containing 15% FCS. The suspension was poured into a Petri dish and was incubated at 37°C for 60 min in 5% CO₂. Then this was washed away with PBS three times and the monocytes adherent to the bottom of Petri dish were removed with a 2:1 mixture of 2% lidocaine hydrochloride and RPMI 1640 containing 15% FCS. Removed monocytes were resuspended and washed twice with PBS, then about 0.2 ml of pellet was prepared. The pellet was mixed with 2 drops of diluted serum of blood group AB, consisting of 2 drops of serum and 1 ml of PBS. Then the mixture was taken on a slide glass. After fixation in cooled buffered formalin aceton at 4°C for 10 min, this preparation was
washed in water for 20 min and stained by the 'simultaneous coupling azo dye method' (Pearse 1972). After the counter staining with 1% toluidine blue 0, microscopic examination was made. Two hundred of monocytes were counted. According to the strength of staining, the cells were subdivided into three groups; diffusely red brown cells (+), faintly red brown cells (±), and not stained cells (–).

**RESULTS**

The level of NBT reduction of monocytes (Fig. 1) was high in the fresh cases and tended to be parallel to the stage of disease in the cases of steroid sensitive INS group. It was normal in the cases of steroid non-sensitive group. These tendencies were not applied to other examinations of monocytes.

IB phagocytosis of monocytes (Fig. 2) was high in the fresh cases of steroid sensitive INS, was normal in the cases of relapse and tended to be low in the cases of incomplete or complete remission. It tended to be low in the cases of steroid non-sensitive group.

Yeast phagocytosis of monocytes (Fig. 3) tended to be high in the fresh cases of steroid sensitive group, and was not consistent in other groups.

Chemotaxis of monocytes (Fig. 4) tended to be low in the fresh cases of the

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**Fig. 1.** Nitroblue tetrazolium dye test of monocytes in INS. Controls n=13, mean±s.d. =12.2±2.5%. o, no proteinuria, no steroid therapy; Δ, no proteinuria, on steroid therapy; o, proteinuria, no steroid therapy; Δ, proteinuria, on steroid therapy.
Fig. 2. IB phagocytosis of monocytes in INS. Controls \(n=13\), mean±s.d. = 74.4±3.3%.

Fig. 3. Yeast phagocytosis of monocytes in INS. Controls \(n=13\), mean±s.d. = 79.9±3.3%.
steroid sensitive group, and to be high in the cases of relapse group and of steroid non-sensitive group. It was not consistent in other groups. Thus the dissociation between chemotaxis and both NBT reduction and TB phagocytosis was observed in the fresh cases of steroid sensitive group and in the steroid non-sensitive group.

Random migration of monocytes (Fig. 5) tended to be low in the fresh cases of steroid sensitive group and to be high in the steroid non-sensitive group.

Chemotactic index (Fig. 6) tended to be low in the fresh cases of steroid sensitive group.

ANA staining of monocytes showed various staining patterns in the controls of children and adults. Examination in our cases failed to reveal any difference in staining pattern between the disease and normal controls.

**DISCUSSION**

While many lines of evidence, including abnormalities of T cell function, have suggested an immunopathogenetic mechanism of INS, there have been few reports on the monocyte function in INS. Since the monocyte has been recognized as a precursor cell of the reticuloendothelial system (RES) (Van Furth 1972), which plays a central role in the induction of immune response with respect to the presentation of antigen to T cells, the comprehensive examination of monocyte function in INS is important.
function will be necessary for further evaluation of cell mediated immunity and RES function in the pathogenesis of INS.

We investigated the function of monocytes of peripheral blood from patients with INS in reference to NBT reduction, phagocytosis, chemotaxis, and ANAE staining.

NBT reduction is concerned with one of the metabolic responses of monocytes, which represents hexose monophosphate shunt activity (Pachman et al. 1973; Cohn 1978). The increase in value of NBT reduction in the fresh cases suggests the stimulation of monocytes by some antigen as described in our previous report (Nagata et al. 1981).

The phagocytic activity was studied with two methods. One using IB, which was coated with anti-IgG antibody, is concerned with IgG-Fc receptor related phagocytosis. The other with Baker's yeast is concerned with non-specific phagocytosis of monocyte. Phagocytosis may depend on the characteristics of the phagocytosed substances and on the condition or the specificity of the surface receptors of monocyte. The reason for dissociating results of the fresh cases of steroid sensitive group and the steroid non-sensitive group

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**Fig. 5. Random migration of monocytes in INS.** Controls n=13, mean±s.d.=0.471±0.112 mm.
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is unknown. Increased phagocytosis in the fresh cases of steroid sensitive INS may reflect activated monocyte function produced by the antigen stimulation or by lymphokines such as macrophage activation factor (MAF), which has been considered as the same substance as migration inhibition factor (MIF) by many workers. It is interesting that the decreased chemotaxis was also demonstrated in the fresh cases of steroid sensitive INS. The decreased phagocytosis in the steroid non-sensitive group may be brought about by the blocking or the exhaustion of monocyte surface by some antigen or immune complexes.

Chemotactic activity is one of the important functions of monocyte. An abnormality of chemotaxis has been observed in several conditions including influenza infection, herpes zoster infection, and neoplasms (Ralph and Mergenhagen 1976). A decreased chemotaxis was observed in the fresh cases of steroid sensitive group, whereas an increased chemotaxis was observed in the steroid non-sensitive group. It is also possible that the monocyte loaded with some antigen or immune complexes may lead to decreased chemotaxis. Meltzer and Stevenson (1978) observed that decreased chemotaxis was accompanied by increased phagocytosis in macrophages of neoplasm-transplanted mice. A similar mechanism may be induced in the fresh cases of steroid sensitive INS. Inversed results were obtained in the steroid non-sensitive group in reference to IB phagocytosis and chemotaxis. The increased chemotaxis may be explained by the monocyte chemotactic factor (MCF), which possibly induces the migration and accumulation of monocytes or promonocytes to the site of delayed type
hypersensitivity. The decreased phagocytosis cannot be explained by MCF or other known lymphokines. There are some studies demonstrating a decreased yeast phagocytosis of monocytes in systemic lupus erythematosus and rheumatoid arthritis (Svensson 1980a, b). It is possible that the increased chemotaxis as well as the decreased phagocytosis of monocytes may be related to renal proliferative changes, since most of our cases of steroid non-sensitive group showed MPGN. It is interesting that many investigators have revealed the participation of monocytes in glomerulonephritis (Atkins et al. 1976; Schreiner et al. 1979; Monga et al. 1979; Holdsworth et al. 1980).

By the simultaneous investigations of the monocytes of peripheral blood, an increased phagocytosis and a decreased chemotaxis were observed in the fresh cases of steroid sensitive INS which showed minimal nephrotic change, while a decreased phagocytosis and an increased chemotaxis were observed in steroid non-sensitive INS which showed mostly MPGN. These findings may suggest different pathogeneses between steroid sensitive INS and steroid non-sensitive INS. Summing up our discussion, we postulate a schema as a possible explanation for the results (Fig. 7).

Fig. 7. A possible explanation of the dissociation between phagocytosis and chemotaxis in the fresh cases of steroid sensitive INS and the steroid non-sensitive INS (MPGN).

MAF, macrophage activating factor; MIF, migration inhibition factor; MCF, monocyte chemotactic factor.
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References


