Alkaline Phosphatase in Neutrophil Leukocytes of Patients with Infectious Mononucleosis and the Effect of Corticosteroid Therapy

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INFECTIOUS MONONUCLEOSIS is not always easy to diagnose, particularly in its initial stages. The hematologic and serologic findings are often negative and the clinical picture may resemble a number of other diseases. This paper is an attempt to apply the knowledge assembled previously on leukocytic alkaline phosphatase to the problem of the differential diagnosis of mononucleosis.

The enzyme alkaline phosphatase in the cytoplasm of neutrophil leukocytes (NLAPA) participates in many important metabolic processes within the cell, particularly during the synthesis and breakdown of glycogen. Healthy subjects ordinarily show a small amount of certain portions of this enzyme in neutrophil leukocytes. NLAPA reacts readily by quantitative changes not only to various diseases affecting the hematopoietic and reticuloendothelial system but also to various physiologic and pathologic conditions of the organism. The estimation of NLAPA used as a diagnostic test in hematology for differentiating granulocytic leukemia from other myeloproliferative syndromes and polycythemia vera from secondary erythrocytosis has recently also been used for diagnosing early pregnancy,5,6,8,24,25 for the detection of acute surgical inflammations,37 for the differentiation of infectious hepatitis from obstructive extrahepatic cholestatic processes and cirrhoses.9,15,18 Beisel,1 King et al.14 and others have published papers on the significance of NLAPA in the diagnosis of hypophosphatasia.

Highly increased values of NLAPA have been observed by Wachstein24 in 1946 and later by Valentine et al.29,33 in inflammatory processes and in different types of infections. Recent information on this subject again emphasizes the fact that only pyogenic infections may lead to a significant increase of NLAPA while the values remain normal or even below normal in some virus infections.1,0,13,15,18,19,35,36

Valentine, Follette, Lawrence and Beck32,33 and others have studied the changes of NLAPA after the administration of corticoids. As corticoids may be very important in the treatment of severe cases of infectious mononucleosis, we studied their effect on NLAPA in our patients.
METHODS AND MATERIALS

The normal values of NLAPA were determined in 22 healthy adults and 20 children aged from 11 months to 11 years. In 30 patients with infectious mononucleosis (aged 7–22 years) the clinical diagnosis was confirmed by hematologic and serologic findings; in the differential blood count the average number of a typical lymphoid monocyte was 11 per cent, Ericson’s test—i.e., hemolysis of bovine erythrocytes with the patient’s serum—was positive with an average titer of 1:675. Blood for the examination of NLAPA was taken from all patients within 24 hours after admission to the hospital, then after intervals of 24 hours and later after 2 or more days up to the day of discharge from the hospital. Moreover, control examinations were carried out in 20 out-patients after 1–9 months, when they were in a satisfactory condition and with no complaints.

In 32 patients from 2–64 years old, suffering from follicular or pseudomembranous tonsillitis of bacterial origin, the most frequent cause of the disease were beta-hemolytic streptococci, pneumococci and the Staphylococcus pyogenes aureus hemolyticus. The clinical picture in these patients did not suggest infectious mononucleosis which was ruled out also of hematologic and serologic examination. Blood for estimation of NLAPA from these subjects was first taken during the acute stage. Some of them were repeatedly examined prior to their release.

The group with an obscure etiology of the disease included 6 very young boys 1.5–5 years old—when the number of a typical lymphoid monocyte was very small and the serologic findings were limited within the range of normal values.

The effect of corticotherapy was studied in another group of 35 patients with infectious mononucleosis (20 of whom were treated with corticoids) and in 5 patients with other diseases. The corticoids were, on an average, administered for 6 days either per os (prednisone 0.5–1 mg./Kg. per day, or a corresponding dose of triamcinolon), or intramuscularly as a cortisone acetate in daily doses of 100–300 mg. according to age of the patient. In addition tetracycline and aureomycin were given to all patients while the individuals with infectious mononucleosis were put on a hepatic diet and were also given vitamins. Blood samples were taken from all patients before steroid therapy, then within 1–6 hours after the onset of treatment, later in intervals of 24 and finally 48 hours. We continued in our investigations even after the corticotherapy had been finished.

PROCEDURE

Alkaline phosphatase was determined by Kaplow’s\textsuperscript{11} semiquantitative cytochemical azo-dye coupling method. The principle of this reaction is that NLAPA in an alkaline medium splits off from a suitable substrate alpha-napthol which, in a coupling reaction with various diazotates, produces its own azo-dye and forms an insoluble precipitate at the site of its origin. In our modification we used a different buffer (Michaels’ veronal acetate with a PH of 9, 4) and an incubation period prolonged to 45 minutes at a temperature of 37 C. The nuclei of the leukocytes were stained with a 2 per cent aqueous solution of methyl green.

The positivity of the reaction was evaluated by a scoring technic after Kaplow\textsuperscript{11} whereby the resulting index of the smear, the so-called “score” can be from 0–400. This method has to be carried out very accurately to avoid many errors leading to unreliable results. It is most important to evaluate the blood smears immediately because of the rapid drop of the enzyme level. The incubation solution should always be fresh. At least 2 smears of a known score must be stained simultaneously and only the middle portion of perfect, thin smears should be evaluated. The percentage of rods in the cells which are being evaluated should not exceed 10 per cent. To avoid subjective errors, the evaluation should be made by 1 worker.

RESULTS

The average value of NLAPA in the normal control subjects was $M = 35.6$ with a standard deviation of $\sigma = \pm 23.1$ (fig. 1). The values were the
same in children and women ($M = 40.8 \pm 24.5$ and $M = 40.0 \pm 22.3$), in men they were slightly lower ($M = 25.6 \pm 17.7$). These data are in keeping with the normal values published in the literature where the widest range of normal NLAPA values is from about 5–100 when applying the same method.

In all patients with acute bacterial tonsillitis NLAPA was increased (fig. 1). In the majority of them the values were many times higher than in healthy subjects. The average value $M$ in this group was $212.2 \pm 51.4$ which is close to the value found, for example, in our patients with purulent meningitis. Two of the patients with diphtheria of the tonsils have not been included in figure 1, because they were examined shortly after we had finished this paper. NLAPA in these patients was 240 and 258 respectively.

In patients with infectious mononucleosis NLAPA was very low ($M = 13.2 \pm 15.5$) and negative reactions were observed in nearly one third of them (fig. 1). This occurred even in such instances where the absolute numbers of granulocytes was as high as in patients with bacterial infections.

In very young patients with a doubtful etiology of the disease NLAPA was slightly above normal but was normal at the time of their release.

The dynamics of NLAPA in the course of the disease is summarized as follows: In patients with bacterial tonsillitis a decline of the reaction was observed in the neutrophils proportional to the regression of the disease reverting to normal levels between the sixth to the thirteenth day. A further increase of the enzyme has sometimes been observed on the following days in such instances where we recognized the disease at its very beginning. This curve is demonstrated on figure 2. Contrary to that, a slight increase of NLAPA was observed in some patients with infectious mononucleosis shortly before their release from hospital, whereas NLAPA of the other patients remained unchanged. In control examinations during convalescence we examined 20 out-patients 1–9 months later. NLAPA values of 18 of these patients were higher then during their illness (fig. 3).

Our results pertaining to the effect of corticotherapy on NLAPA in infectious mononucleosis are given in figure 4. Sixteen of 20 patients showed increased enzyme values in leukocytes (30–1700 per cent above the original value). Figure 4 shows that in patients to whom the corticoids had been given per os, the NLAPA increase is slower with the peak usually 24–48 hours after the onset of treatment, while the peak is reached earlier, sometimes even after 1 hour, when given intramuscularly. Since our examinations were carried out only in a few patients the differences in the effect of the individual preparations could not be estimated.

In 5 patients examined shortly after the administration of the first dose of corticoids, we observed a brief negative course of the curve (fig. 5). No negative phase was noted in the other patients or its course was too quick to be recorded.

No increase of the enzyme activity in the leukocytes was observed in 4 patients after the administration of corticoids.

In our control group of 15 patients with infectious mononucleosis not treated with corticoids, we found only in 1 female patient, treated with
Fig. 1.—NLAPA values of the individual patients after admission to hospital: 1st column. Patients with infectious mononucleosis and a negative NLAPA: no value given (in the right part of the column). The horizontal stripe crossing the figure: average value M and the mean deviation sigma in normal persons.

Fig. 2.—NLAPA dynamics in streptococcal lacunar tonsillitis in an 8-year-old girl. Admission on the first day of the illness. A further slight increase of enzyme in the leukocytes during the following 4 days, then a sudden decrease; she was discharged from hospital after 10 days. The values of the day of release were close to the values of the control examination 23 days later.

tetracycline, a NLAPA curve which, to a certain degree, resembled the curves observed after corticotherapy.

The effect of a superinfection on NLAPA in infectious mononucleosis could be compared with the effect of cortisone in 3 patients, of whom 2 acquired an acute respiratory infection and 1 adnexitis. Figure 6 shows...
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Fig. 3.—Differences in NLAPA values in the acute stage of infectious mononucleosis and the control examination 1–9 months after recovery. Black column—NLAPA in the acute stage; white column—during convalescence. Patients with negative NLAPA in the acute stage—only white columns. The figure shows that NLAPA of most patients is much higher during convalescence than during the disease.

NLAPA curve of 1 of them. In all 3 instances, the increase of the enzyme is far more marked in secondary infections than after the administration of corticoids.

The effect of the corticotherapy on NLAPA in diseases with increased original values is shown in figure 7. Four of these patients had purulent bacterial tonsillitis, 1 of them stomatitis. The figure shows that their NLAPA decreases with the recording disease in spite of the cortisone treatment.

DISCUSSION

Our findings in bacterial tonsillitis are generally in agreement with previously published data of an increase of NLAPA in various pyogenic infections and bacterial inflammations.11,18,19,20,34,35,37

There is little information available regarding the findings in infectious mononucleosis. Whenever this disease is mentioned in the literature the authors found either low or normal NLAPA values.1,9,13,14,36 Only Tanaka et al.26 studied a greater number of patients, using the biochemical determination; in some of the patients the enzyme levels were decreased, in others normal. A return to normal of these reduced values was observed only 8
Fig. 4.—A survey of the effect of corticoids on NLAPA in patients with infectious mononucleosis. Dark columns—NLAPA values before administration of hormones; light columns—increase of the absolute score values. Indication of the highest value during therapy and the time interval of the increase to the peak (numbers under the corresponding columns).

Fig. 5.—Effect of corticotherapy on the NLAPA in a patient with infectious mononucleosis. The first part—intervals in which the blood was taken from the patients, given in hours; the second part—in days. A brief primary decrease followed by marked, prolonged increase (transient).

weeks after the onset of the disease. Hausser and Strohn also report a decrease of the average NLAPA value in fifteen patients. However, closest to our own results are the data of Lambers from 9 patients with a decreased or negative NLAPA.

Our studies of patients recovering from infectious mononucleosis indicated that in most patients NLAPA did not increase during the acute phase of the
disease but that its values were below normal despite this acute stress situation. During convalescence the amount of enzyme increased gradually. Since infectious mononucleosis affects mostly the reticuloendothelial system, our findings are not in agreement with the hypothesis of Keiser and Alseleben\cite{12} (1963) according to which all diseases affecting this system tend to increase NLAPA values. Another exception mentioned by these authors is paraproteinemic reticulosis.
In most of our patients with infectious mononucleosis, we observed a marked activation of NLAPA after the corticotherapy; similar observations were made by Trubowitz and Valentine\textsuperscript{27,32,33} after the administration of ACTH and cortisone to normal persons. An even greater increase of the enzyme in the leukocytes occurred in several subjects a superinfection with another infective agent. Apparently, in the majority of cases with infectious mononucleosis, not the inability of the granulocytes to react to the infective stimulus is involved but only inactivity of the mechanism leading to the activation of the neutrophils in other infectious processes, or these mechanisms are inhibited in some way. There is, therefore, no analogy with leukocytes in chronic myeloid leukemia where the NLAPA values are also low but do not increase significantly after the administration of cortisone during infections. This fact has also been confirmed by most authors\textsuperscript{4,7,10,17,18,21,26,32} and appears to be a fundamental difference indicating various types of a low NLAPA.

**Conclusion**

A marked increase in the activity of the neutrophil alkaline phosphate enzyme (NLAPA) was observed in bacterial tonsillitis whereas in infectious mononucleosis the amount of enzyme in the leucocytes was either decreased or normal. NLAPA of patients with tonsillitis decreased in proportion to the receding disease; in infectious mononucleosis the enzyme increased during convalescence. In 16 of 20 patients with infectious mononucleosis, NLAPA increased after corticotherapy. The value of the enzyme in patients with infectious mononucleosis was much higher after superinfections caused by a different infective agent than after corticotherapy. The studies demonstrate the value of NLAPA examinations in the early diagnosis of infectious mononucleosis, carried out during the acute febrile state and especially during the first days of the illness when all other laboratory examinations may still give negative results.

**Summario in Interlingua**

Un marcate augmento del activitate de neutrophilic phosphatase alcalin esseva observate in casos de tonsillitis bacterial durante que in mononucleosis infectiose le quantitate del enzyma in le leucocytos esseva reducite o possibilemente normal. In patientes con tonsillitis le activitate del enzyma declinava in correlation con le recession del morbo. In mononucleosis infectiose le quantitate del enzyma cresceva durante le convalescentia. In 16 de 20 patientes con mononucleosis infectiose, le activitate de neutrophilic phosphatase alcalin cresceva post therapia a corticosterone. In patientes con mononucleosis infectiose, le nivello del enzyma esseva multo plus alte post le superimposition de un infection causate per un altere agente que post therapia a corticosterone. Le studios hie reportate demonstra le valor de tests del activitate de phosphatase alcalin in le leucocytos neutrophilic in le precoce diagnose de mononucleosis infectiose, effectuate durante le acute stato de
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febrilitate e specialmente durante le prime dies del morbo quando omne altere examines laboratorial produce generalmente resultatos negative.

REFERENCES
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