

From the Allergy Department of Rothschild Foundation, Paris, France.
Head: P. Gervais, M.D.

THE VACUOLISATION OF EOSINOPHILS AND ITS SIGNIFICANCE IN THE SKIN-WINDOW TEST

By

T. DIKEAKOU, E. GARELLE & P. GERVAIS

The eosinophil being a cell of the myeloid group is endowed with phagocytic function as are the other cells of the same group (*Speirs & Speirs* (18) *Archer* (1)).

Its presence in an antigen-antibody ($Ag + Ab$) reaction was considered to be due to its histamine content or to its attraction by it. Later studies though have demonstrated that the role of eosinophils in an immune reaction is different and much more important. It was shown that the role of the eosinophil in the immune process is an active one. *Cohen* (3, 4) *Litt* (12, 14) *Roberts* (16) have demonstrated that the eosinophil plays a great role in the primary reaction of immunisation by phagocytizing and transporting the Ag to the regional lymphatic glands with little delay (4h).

The same authors as well as *Sabesin* (17) have shown by electron microscopy that the role of eosinophils is of the same importance in the secondary reaction. In this case it phagocytizes the $Ag + Ab$ complex (5, 6, 9, 11, 15). Recent work by *Kostage*, *Rizzo* & *Cohen* (9) demonstrates that the phagocytic capacity of the eosinophil is not limited to the $Ag + Ab$ complex but is referred to any substance having a certain molecular configuration and molecular weight. *Litt* (11) has shown by fluorescent technique that this phagocytosis appears as cytoplasmic vacuolisation. *Connel* (16) in a recent work demonstrates that the blood eosinophils present a con-

siderable vacuolisation in *allergic patients* as well as of patients suffering from a disease with tissue necrosis.

The percentage of vacuolated eosinophils in these cases is much higher than that of patients suffering from other diseases and that of apparently healthy controls. He found vacuolisation of eosinophils in 40 per cent of allergic symptomatic patients.

Connel considers the vacuolisation as the phagocytosis of the specific Ag + Ab complex or of the Ag alone in the blood of allergic patients, while in other cases as the phagocytosis of a foreign or a denatured protein derived from the inflammatory process of the disease, not excluding the possibility of a phagocytosis of an autoimmune complex. We thought that the skin window technique was appropriate for the study of this phenomenon, because we can study the cellular response to a given antigen. Possibly a study of the vacuolisation could give a specific significance to the skin window eosinophilia and be helpful to illustrate the physiological function of eosinophil.

MATERIAL AND TECHNIQUE

Our material consists of 45 skin window tests (S.W.T.) with eosinophilia. This eosinophilia was the response to the application of antigens of different chemical nature, i.e. proteins, polysaccharides with small admixture of protein, and haptens. The eosinophilia varied between 3-50 per cent. We defined an eosinophil as a cell with two or exceptionally three, nuclei and orange coloured cytoplasmic granulation. The slides were read after May-Grünwald-Giemsa staining and under Wild M11 microscope. The vacuolisation was related 1) to the percentage of eosinophilia in the S.W.T. with Ag and 2) to the percentage of eosinophilia in S.W.T. controls and 3) to the factor of time. We considered an eosinophil vacuolated if at least 3 vacuoles were present in the cytoplasm, while Connel counted only two.

RESULTS

The statistical studies have shown that eosinophilia is independent of time. The mean value being of the order of 13 per cent did not show any specific trend until the 24th hour ($p < 0.001$) and 8.2 per cent the 48th hour with a probability

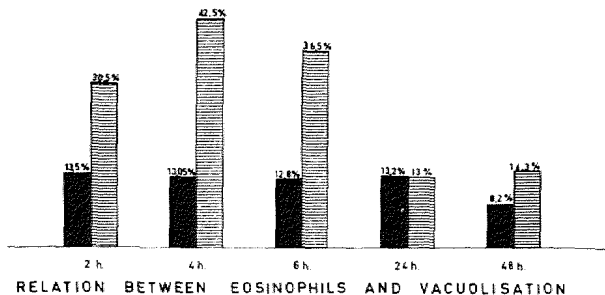


Fig. 1.

Percentage of vacuolisation in relation to the time. Black colour: eosinophils. Shaded: vacuolisation.

of error less 2.5 per cent (Fig. 1). On the contrary the vacuolisation shows a specific trend with a peak (42.5 per cent) at the 4th hour after which it declines. There is no threshold of vacuolisation. The probability of vacuolisation appearing in the control is less than 0.05 per cent while in the S.W.T. with Ag it is higher than 83 per cent.

DISCUSSION

Considering the two parameters i.e. eosinophilia and vacuolisation we feel that the specific phenomenon is the vacuolisation and not the eosinophilia, which has not a specific trend. The eosinophilia can be the response to an Ag + Ab complex as well as to a nonspecific mechanism, for example (1) to the histamine liberated during the lesion of the skin before the Ag + Ab reaction takes places, (2) to the substances liberated during this reaction, or (3) to a non-specific inflammation due to the substance tested. The vacuolisation is the expression of cellular phagocytosis. It may be due to the phagocytosis of an autologous, denatured protein, either of the Ag itself, or of a complex Ag + Ab. The specific trend and its dependence on time suggests the phagocytosis of an Ag + Ab complex of the immediate type.

It seems reasonable to consider as a specific response of S.W.T. the vacuolisation rather than the eosinophilia which may be a non-specific response. Thus in 7 S.W.T.s with eosinophilia after the application of a hapten only one showed a vacuolisation of 10 per cent. We wonder whether in the other cases non-specific factors i.e. histamine, denatured tissue proteins can cause eosinophilia but not on Ag + Ab reaction.

Thus it is evident that a S.W.T., with eosinophilia can present itself in three forms.

- (a) with an eosinophilia completely vacuolated
- (b) with an eosinophilia partially vacuolated
- (c) with an eosinophilia not at all vacuolated

It is not difficult to accept the positivity of the two first forms. Concerning the third one, one wonders if an Ag + Ab reaction takes place unless we accept that there is an Ag + Ab in excess which would inhibit the phagocytosis of a stable complex of precise configuration, although *Litt* (14) accepts that a complex with an excess of Ag may be phagocytized.

CONCLUSION

Our results are in agreement with those of Connel. We have found the vacuolisation to reach 42.5 per cent counting 3 vacuoles in the cytoplasm of the eosinophil while *Connel* (8) considered a vacuolisation very abnormal if it was above 50 per cent and moderately abnormal between 30–10 per cent counting two vacuoles per cell.

Also, in the drug reactions Connel did not find any vacuolisation and neither did we when we used a hapten (drug).

The use of the S.W.T. with the specific Ag has doubled the frequency of the vacuolisation. Thus in Connel's study, vacuolisation was present in 40 per cent of the cases while in our study it was higher than 83 per cent. This confirms the specific nature of the vacuolisation as suggested by Connel.

Apart from the significance one can attribute to the vacuolisation in relation to the positivity and specificity of S.W.T.,

one can also obtain information about the physiological function of the eosinophil. It seems to be a cell which is attracted by certain factors aimed at the elimination of the $\text{Ag} + \text{Ab}$ complex and of the substances derived from the inflammation. In this way it participates in the limitation of the inflammation *Archer* (2).

The study of the vacuolisation in the eosinophilia of S.W.T. with a given Ag supports on one hand the theory that histamine can potentiate the cellular response, and on the other hand the necessity for the phagocytosis of a substance with a certain configuration and molecular weight. This may be assumed from the fact that some eosinophils are not vacuolated and consequently are attracted but not used.

From the practical point of view a S.W.T. read after six hours which has presented an eosinophilia with vacuolisation higher than that of the control, may be considered a response of the immediate type reaction towards a given Ag. A vacuolisation in the control is an abnormal phenomenon which one does not expect to find. Thus when it appears it needs an explanation.

Concerning the non-vacuolated eosinophilia, even if it does not represent an $\text{Ag} + \text{Ab}$ complex, it is clinically significant because the clinical manifestations may be of allergic or par-allergic origin *i.e.* due to a specific or a nonspecific histamine release.

The physiological function of the eosinophils in relation to the elimination of inflammation, their presence in the S.W.T. not strictly bound to the $\text{Ag} + \text{Ab}$ complex, as well as the specificity of the vacuolisation, are shown by their evolution in relation to time.

In readings at 24 and 48 hours, the mean values of eosinophilia remain high, while those of vacuolisation are very low. This means that when one expects a reaction ($\text{Ag} + \text{Ab}$) of the immediate type to be maximally inhibited, the eosinophilia remains high in contrast to the vacuolisation which will be reduced.

SUMMARY

Litt has demonstrated that phagocytosis of the Ag + Ab complex appears as a vacuolisation in the cytoplasm of eosinophils. *Connel* has shown that 40 per cent of allergic patients with clinical manifestations, show a vacuolisation of the eosinophils in the blood.

In our material consisting of 45 skin window-tests with eosinophilia, the statistical study has shown the presence of vacuolisation in 83 per cent with a specific trend in relation to time. The vacuolisation reaches its peak at the 4th hour. The eosinophilia remains at a constant low level (13-8 per cent).

REFERENCES

1. *Archer, G. T. & Hirsch, J. G.*: J. Exp. Med. 118, 287, 1963.
2. *Archer, R. K.*: British J. Haemat. 11, 123, 1965.
3. *Cohen, S., Vassali, P., McClunsky, R. C. & Benacerraf, B.*: Fed. Proc. 24, 696, 1965.
4. *Cohen, S. G., Kanmor, M. & Catto, L.*: J. Allergy 32, 214, 1961.
5. *Cohen, S. G., Sapp, T. M. & Gallia, I. R.*: Proc. Soc. Exper. Biol. and Med. 113, 29, 1963.
6. *Cohen, S. G., Sapp, T. M., Rizzo, A. P. & Kostage, S. T.*: J. Allergy 35, 346, 1964.
7. *Cohen, S. G. & Sapp, T. M.*: J. Allergy 36, 415, 1965.
8. *Connel, J. T.*: J. Allergy 41, 1, 1968.
9. *Kostage, S. T., Rizzo, A. P. & Cohen, S. G.*: Proc. Soc. Exper. Biol. and Med. 125, 413, 1967.
10. *Kline, S. B., Cohen, M. G. & Rudolph, J. A.*: J. Allergy 3, 531, 1932.
11. *Litt, M.*: J. Cell. Biol. 23, 355, 1964.
12. *Litt, M.*: Am. J. Pathol. 42, 529, 1963.
13. *Litt, M.*: J. Allergy 33, 532, 1962.
14. *Litt, M.*: Annals N.Y. Acad. Scienc. 116, 964, 1964.
15. *Patterson, R., Suszka, J. M. & Pruzansky, J. J.*: J. Immunol. 80, 471, 1962.
16. *Roberts, A. M.*: Nature 210, 266, 1966.
17. *Sabesin, S. S.*: Proc. Soc. Exp. Biol. and Med. 112, 667, 1963.
18. *Speirs, R. S. & Speirs, C. E.*: J. Immunol. 90, 561, 1963.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.