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Allergic inflammation, its development mechanism and per-meability of cell membrane

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Allergic inflammation, its development mechanism and per-meability of cell membrane*

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Abstract

The ascitic monocytes and subcutaneous cells and tissues of sensitized animals have been observed after exposing to antigen for the purpose of revealing the disintegration processes of the cells related with inflammation and it has been proved that the permeability of the cell membrane increases markedly resulting in the swelling of the cells at the moment when the cells come in contact with antigen. The localization of the antigen in the Arthus' phenomenon will be the results of the gelatination of the inter-cellular tissues and the swelling of cells. And it is indicated that the cell death accompanied by an allergic inflammation is caused by the increased permeability of the cell membrane which will result in the activation of the intra-cellular enzymes followed by the acute disintegration of the molecular structure of the cell and release of the so-called inflammatory substances.

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ALLERGIC INFLAMMATION, ITS DEVELOPMENT MECHANISM AND PERMEABILITY OF CELL MEMBRANE*

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As has been reported precisely by MENKIN[1,2,3], it is obvious that in allergic inflammations, those substances released from the damaged cells act as the important factors for the development of the inflammation, i. e. infiltration of leucocytes and the acceleration of permeability of the capillaries and cell membrane caused by leucotaxin, obliteration of lymph vessels by fibrin thrombus, infiltration of macrophages by the macrophage promoting factor of POMERAT[4], tissue necrosis by the necrosin of Menkin, leucocytosis by the leucocyte promoting factor and so on. But the damage of cells or tissues does not necessarily result in the inflammation, for instance in the case of oedema or infarcts caused by the simple circulatory disturbances or necrosis by the heavy metal intoxication such as mercury nephrosis where always the cell death occurs but are not followed by the typical inflammation. Therefore, for the development of inflammation followed by tissue damage, it is supposed that there must exist a certain type of cell death by which the inflammation is induced. The typical type of the inflammatory cell damage may be seen in the destruction of tissues by exposing to some inflammatory agent like croton or terpentine oil. But in this case it is not so easy to differentiate the primary damage directly connected inflammation from those being not responsible for the following inflammation.

In the case of allergic inflammation, however, it may be possible to catch essential cell damage which is directly connected to the development of inflammation, because in this case, the tissue damage is supposed to occur only on those cells having antibody. From this viewpoint, the authors observed the initiation of the cell death in antigen-antibody reaction, both on ascitic cells and on the subcutaneous tissues by injecting the antigen to the sentitized rabbits.

The purpose of this paper is to show that the permeability of the

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cells from sensitized rabbit is accelerated immediately after exposing to the antigen, with the mechanism of the damming up of the antigen.

MATERIALS AND METHODS

Adult male rabbits served as materials. These are sensitized by bovine serum introducing subcutaneously 3 cc. every two days five times. And the ascitic cells and subcutaneous tissues have been observed two weeks after the last injection, affecting with or without the antigen. For the morphologic observation of ascitic cells, two to five ml. of the ascites was taken from each animal after the starvation for 24 hours. To each of these is added 1/10 volume of physiologic saline solution containing 0.005 % neutral red respectively and they were divided into two groups. To those belonging to the first group a half volume of bovine serum was added and those belonging to the second group were left without addition of the antigen. All of them were incubated at 37°C for 5—10 minutes and then the cells were observed under the phase-contrast microscope in wet.

The measurement of the permeability of the cells membrane were carried out by using Janus green B or Nile blue. The dyes were taken into four test-tubes 0.1 cc each. Two of them were added with a half volume of bovine serum respectively and another two were left without addition of antigen and these were incubated at 37°C for 30 minutes. Combining one with and the other without bovine serum as a pair, to each test-tube of these two pairs was added five drops of saline solution containing 0.1 % Nile blue and to each of another pair was added the same volume of the same solutions containing 0.1% Janus green B; and all four testtubes were incubated 20 minutes further. Then the cells were smeared, dried and fixed by our method for the fixation of the dyes stained cells supravitally[5,6,7] and the amount of dye immigrated into the cells were measured by the microspectrophotometer.

For the observation of the subcutaneous tissue cells, the abdominal skin of the sensitized rabbits was shaved of the hair, and on one hand the antigen containing Nile blue or Janus green B and on the other rabbit serum mixed with each dye were injected into the subcutaneous tissues respectively in four separate areas and the local reactions were observed by incision. The tissues taken out were extended, dried and fixed by the method just mentioned for the purpose to observe the dyes stained cells supravitally or fixed by 10 % formalin for Van-Gieson and fibrin stainings.

The sera containing the dyes were prepared by mixing 0.5 cc of bovine or rabbit serum with 0.1cc. of 0.1% Nile blue or with 0.25cc. of 0.1% Janus

green B, and they were injected in the volume of 0.6 cc. in the serum Nile blue mixture and 0.75 cc. in the serum Janus green mixture, respectively.

Fixation of the dye stained cells supravitally : The cells stained supravitally by Nile blue or Janus green B were smeared, dried quickly, exposed to formal-vapor for 10 minutes, fixed by potassium mercuric iodide solution which is prepared by mixing 100 cc. of 5 % mercuric chloride with 60 cc. of 1.5 % potassium iodide solution for 5 minutes and washed with tap water. Almost all the dyes used generally for the supravital staining can be fixed by this method. Post-staining of the nucleus may be made by hematoxilin, if required.

Quantitative measurement of the intra-cellular dye concentration : The measurement of the permeability using dye is rather a classical method[8], but this method may be useful to know the changed physico-chemical nature of the cell membrane irrespective of the active permeadility by the action of enzymes, which is generally the case of the metabolites of the cell. But the immigration of dyes into cells is always a function of time and dye concentration and it is impossible to use this for the scale of the measurement of the permeability, when we observe cells in wet[9,10] where the cells have different staining time with each other.

By applying our method, however, we can observe the cells having the same staining time on each slide and the difficulty can be overcome.

Besides these, now we have a method, microspectrophotometric method which has been developed by Caspersson and his collaborators[11,12] by which the dye immigrated into the cells can be measured in each cell. Thus the permeability test using dye takes a new promising step.

To utilize this new method for the measurement of permeability it may be necessary to decide whether or not the dyes used satisfy the Lambert-Beer's law even in a greatly diluted concentration found in a cell and to know the absorption maximum point of the dye which may be changed by forming insoluble complex with potassium mercuric iodide. As is indicated in Figs. 1 and 2 both Nile blue and Janus green B increase their optical density parallel to the increased concentration of dyes in the range of 0.1 to 1.0 %. By fixing with potassium mercuric iodide the absorption maximum of Janus green B changed from 5, 900 Å to 6, 250 Å at pH 7 and 6, 000 Å to 6, 2000 Å in Nile blue (Figs. 3, 4). Therefore, the measurements were taken at 6, 250 Å in Janus green B and 6, 200 Å in Nile blue.

For the measurement of the dye concentration in each cell, the microspectrophotometric apparatus of Olympus Company attached with the microsliding stage designed by SENO and UTSUMI has been used. To minimize the S. V. effect, a spot light of 1.5 μ in diameter was obtained

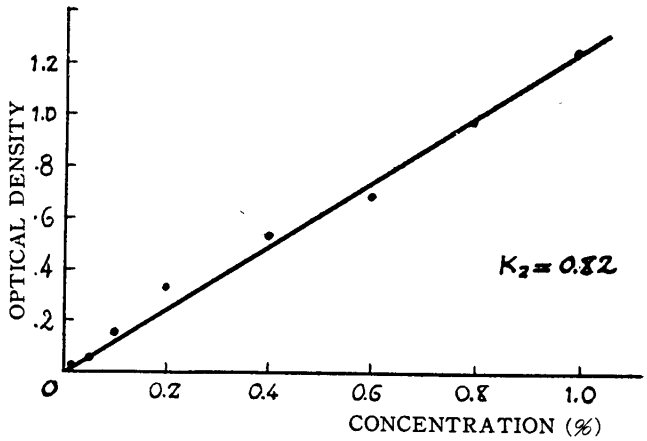


Fig. 1. Optical density determined by the film 30 μ in thickness of 0.2—1.0% Nile blue solution (0.01 cc placed in between an object glass and a slide glass of 1.8 × 1.8 cm). The concentration of dye in the cell being about the same as that of vital staining. The curve runs almost in a straight line. Nile blue in such concentrations follows Lamvert Beer's law sufficiently as to make it possible to determine the dye concentration by measuring the optical density (absorption at 6,000Å). Measurements in each case were taken with a microspectrophotometer of Olympus Co. (light spot of 1.5 μ).

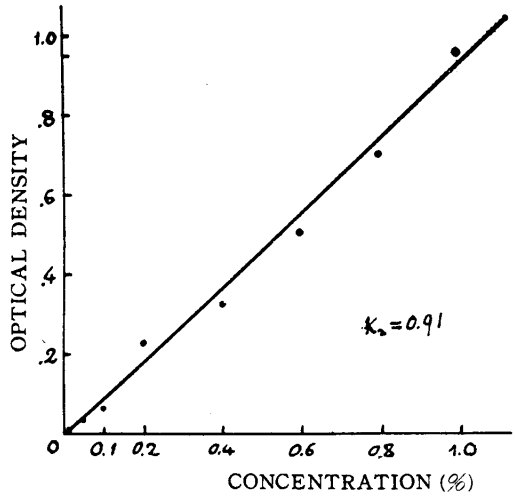
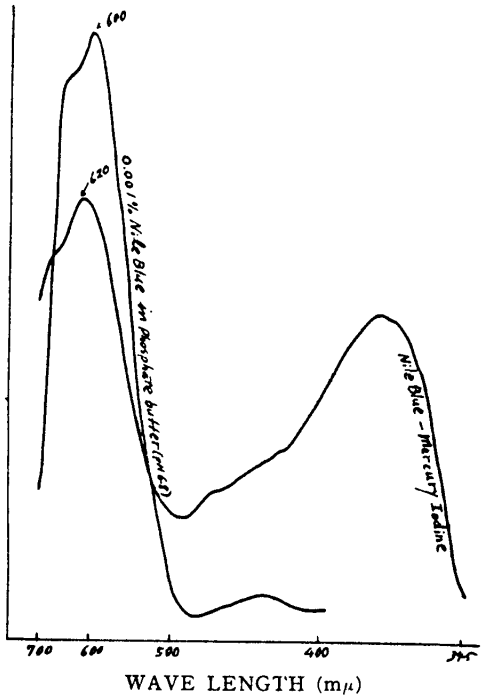


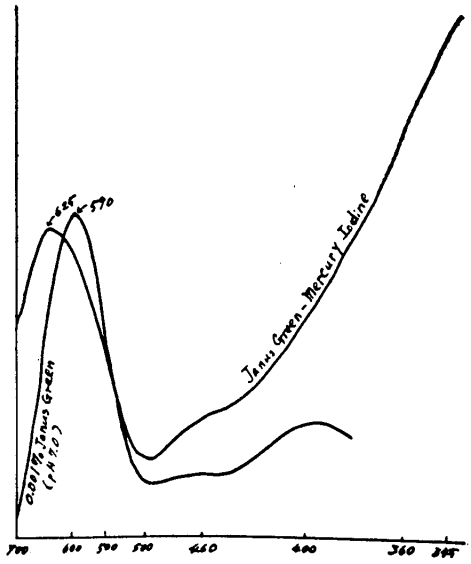
Fig. 2. Janus green, as in the case of Nile blue, shows a similar curve, indicating that it is possible to determine the dye concentration by measuring the optical density (absorption at 5,900Å).

by using objective lens of × 175 as the condenser and only the areas wanted to measure the optical density were illuminated by this spot light[19]. This spot light was moved along the cell diameter and curve $f(x)$ was obtained. The area S obtained by integrating $f(x)$ from o to d (Fig. 5)



WAVE LENGTH (mμ)

Fig. 3. Showing the absorption spectrum of 0.01% Nile blue with its maximum at 6,000Å. The lower one is the absorption spectrum of rabbit serum in which 0.01% Nile blue was dissolved, and smeared on the object glass, fixed with potassium murcuric iodide solution, revealing the optical density to have moved to 6,200 Å with a resultant new peak outside the ultra violet ray (by Beckman DK).



WAVE LENGTH (mμ)

Fig. 4. Showing the change in the maximum optical density of Janus green from 5,900Å to 6,250Å, due to fixation (under the same condition as in the case of Nile blne).

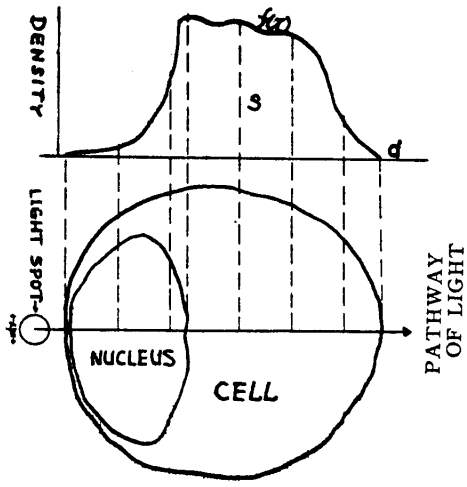


Fig. 5. Showing a diagrammatic method of integration of optical density as measured along the diameter of a single cell for the determination of the dye concentration.

was compared in each cell as the indicators of permeability.

RESUTLS AND COMMENT

Some of the monocytes from the sensitized rabbit were found to be swollen by contacting with the antigen, contracting their pseudopodes and increasing their diameter (Figs. 6, 7), but some of them showed no

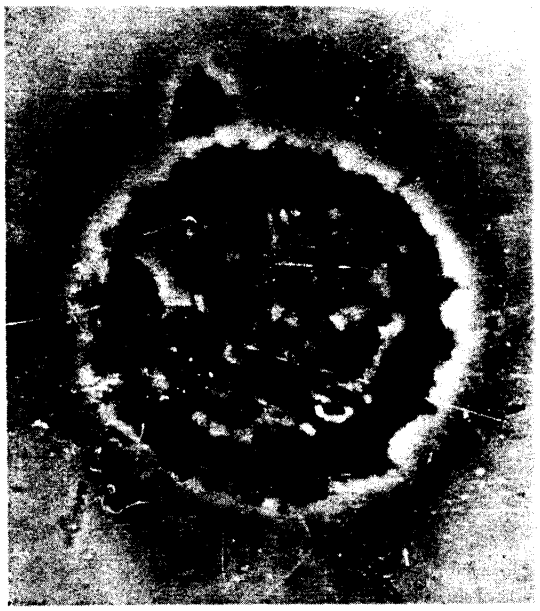


Fig. 6. A phase-contrast microscopic picture of a cell of the rabbit sensitized with bovine serum and supravitally stained with neutral red. Neutral red granules appear black, showing pseudopodes. × 90 × 15 × 3.2 (oil, dark, med.)

reaction to the antigen, neither changing their diameter nor pseudopodes. These reactions occurred similarly *in vivo* as *in vitro*.

The neutral red granules which were recognized in the untreated cells disappeared by contacting with antigen. The dye scattered diffusely in the cytoplasm, suggesting the changes in the electric potentials of the cytoplasm or in the lipid distribution. These findings reaffirmed the data reported by SENO and KAMON[15] and KAMON[13] from the observation on the ascitic monocytes from the sensitized rabbits by using colored antigen or those reported by HAYASHI and others[14,16,17] on the same cells by using egg-albumin as the antigen. The swelling of the cells will be the results of increased permeability of the cell membrane which will be induced by

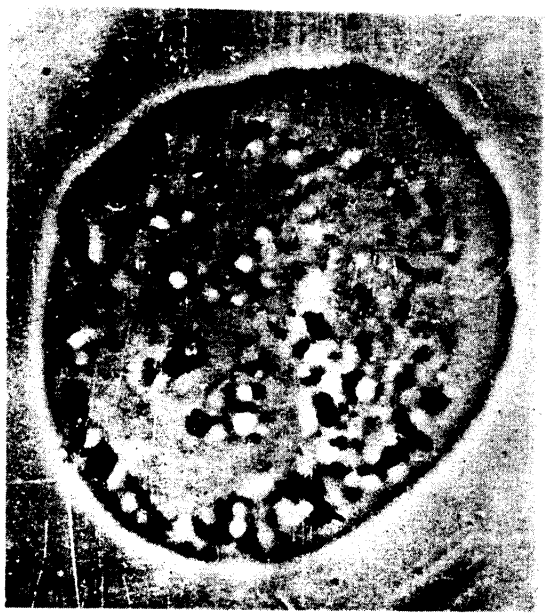


Fig. 7. A phase-contrast microscopic picture of abdominal monocyte of the sensitized rabbit, supravitally stained with neutral red, 20 minutes after the contact with the antigen. Showing a marked swelling of the cell and the loss of pseudopodes and neutral red granules (cf. Fig. 9) × 90 × 15 ×3.2 (oil, dark, med.).

the structural disintegration occurring on the cell membranes as well as in the cytoplasm allowing the intercellular invasion of hydrophilic ions like Na^+, Cl^-, etc. The structural change of cytoplasm can be seen morphorogically as the coagulation of the basophilia[18], when the cells are smeared, dried, fixed and stained by Giemsa. The shedding off of the cytoplasm of monocytes followd by the nuclear disintegration can be seen when the antigen is introduced into the peritoneal cavity of the sensitized rabbits[13].

Actual measurement of the diameter of the swollen cells revealed that the cells affected by the antigen increase in their diameter by about 2.2 to 2.8 μ. The measurement of the optical density at 6, 250 Å in Janus green B and 6, 100 Å in Nile blue on the supravitally stained cells proved that by contacting with the antigen, intracelluler dye concentrations increase showing the increased optical density from 13.6 to 32.6 in the case of Janus green B and from 13.2 to 29.3 in Nile blue (Fig. 8).

This means that the permeability to dyes increases about 2.3 times when the cells are exposed to antigen. The dyes found in those cells af-

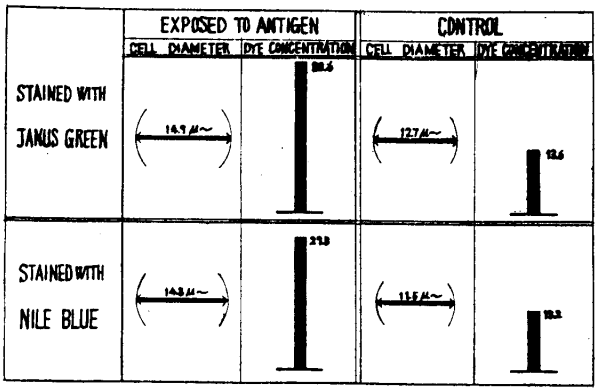


Fig. 8. The left half of the figure shows the average value of 35 monocytes, freely selected, from the sensitized rabbit and exposed to the antigen; and the right half shows the similar average without exposure to the antigen.

fected by antigen are scattered diffusely in the cytoplasm, while in the normal cells the dyes appear granular. This phenomenon does not signify the result caused by an increase in adsorbing power of intracellular substances by the changed ionic milieu irrespective of the increased permeability of cell membrane, because the dyes stained cells supravitally can be removed easily by affecting non-polar solvents like ethanol or methanol after smearing and drying, in the cells exposed to antigen similarly as in the case of untreated cells. This fact proves that the dyes are not combined to any organellae by adsorption, or chemical binding. Therefore, the increased dye concentration found in each cell can be taken as an index of the increased permeability itself.

The subcutaneous introduction of the antigen : It is well known that if the sensitized animals are injected with the homologous antigen, then an inflammation named as Arthus' phenomenon is induced. The subcutaneous injection of the bovine serum mixed with the above mentioned dyes showed that the dyes are localized in the injected area in the case of sensitized animals while in the normal animals the dyes are scattered diffusely and have almost disappeared in 20 to 30 minutes after the injection. The localization of the antigen in the sensitized animals has been explained by MENKIN that this phenomenon is the results of the formation of fibrin nets caused by the imbibition of fibrinogen from the capillaries and the increased activity of the tissue thrombokinase from the damaged cells, and the obliteration of lymph vessels by the fibrin thrombosis similarly as in the case of a general inflammation[1,2,3]. However, if that is the case, it should take some period for the complete localization of

antigen and may show some expansion of the antigen before it is dammed up and yet the fibrin nets could not dam up the diffusion of those substances of small molecules like the dyes. Our observation proved that the damming up of the dyes occurs immediately and promptly after the injection of the antigen and complete preservation of the dyes strictly kept within the injected area showing no diffusion. Therefore, for the localization of the injected dyes, another mechanism rather than the formation of the fibrin nets should be taken into consideration. The resected tissues showed some increase in the rigidity of connective tissues and the extension on an object glass resulted often in the distortion of the tissues, while the tissues injected with rabbit serum proved to be soft and easy to be extended without showing any rigidity. Microscopic observation of the tissues revealed that the increased rigidity is of the gelatinous coagulation of the intercellular substances. This gelatination may be caused by the precipitation reaction between the antigen and the tissue antibody which will be connected to the intercellular structures or derived from the damaged cells. The damming up of the dyes is also seen occurring at the site of the gelatination of tissues where the dyes are detected massively staining the intercellular substances. The cells found in the local area showed a marked swelling and the increased permeability to the dyes, presenting the picture of a deeply stained cytoplasm. This occurs on histiocytes, fibroblasts and monocytes in the tissues. The reactions were almost the same as that of monocytes from the ascitic fluid of sensitized animals.

As it is supposed that the reaction is directly related to the antigen-antibody reaction, these macrophages will have antibody forming in the cytoplasm or taking up from the body fluid. The swelling of these cells occurring by the increased permeability also will play a role in the damming up, but the swelling of the cells only could not dam up the stream of tissue fluid completely, because the subcutaneous tissues at the abdominal wall is rather loosely constructed.

From these findings it seems that the onset of the Arthus' phenomenon will initiate the swelling of cells and the gelatination of intercellular tissues and be followed by the cell infiltration caused by the release of inflammatory substances from the damaged cells and tissues, showing that inflammatory cell death may be the specific one due to acute disintegration of the cells, which will be caused by the destruction of cell membrane with the increased permeability and the activated intracellular enzymes.

CONCLUSION

The ascitic monocytes and subcutaneous cells and tissues of sensitized animals have been observed after exposing to antigen for the purpose of revealing the disintegration processes of the cells related with inflammation and it has been proved that the permeability of the cell membrane increases markedly resulting in the swelling of the cells at the moment when the cells come in contact with antigen. The localization of the antigen in the Arthus' phenomenon will be the results of the gelatination of the inter-cellular tissues and the swelling of cells. And it is indicated that the cell death accompanied by an allergic inflammation is caused by the increased permeability of the cell membrane which will result in the activation of the intra-cellular enzymes followed by the acute disintegration of the molecular structure of the cell and release of the so-called inflammatory substances.

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