The Origin of Monocytes in the Spleen

P. BUENO

Department of Pathology, Biological Institute, São Paulo, Brazil

In recent studies on the reactions of the RES to various antigens and during infectious processes, observations were made by the writer which seem to indicate the site of origin of monocytes in the spleen. These observations were made in guinea pigs which had been inoculated with bacterial toxins or which were carriers of experimental brucellosis.

The histological study of the spleen in these cases showed peculiar reactions which were characterized by regressive or proliferative changes in the lymph follicles and by the presence of numerous mononuclear cells in the sinuses of the pulp.

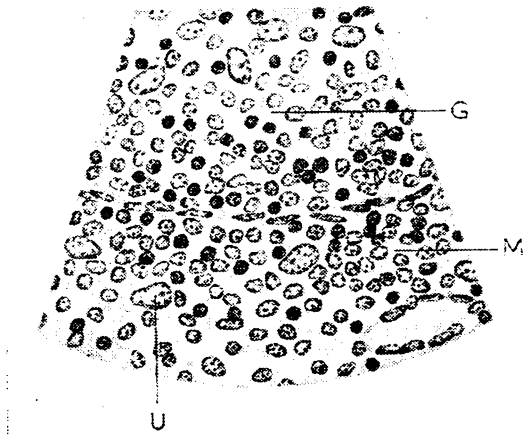

FIG. 1. Sector of a Malpighian corpuscle: G, germinal center; M, marginal zone; U, undifferentiated cell. Hematoxylin-eosin stain. (About 700 X.)

These mononuclears may be regarded as being perfectly identical with monocytes or macrophages. Generally they have a large, rounded or kidney-shaped nucleus with scarce chromatin, and slightly acidophile and well-developed cytoplasm. In the case of *Brucella* infection they appear in great numbers showing marked phagocytic activity, their cytoplasm containing red blood cells as well as cell particles and granules of hemosiderin.

What seemed particularly interesting, however, was the fact that, according to our findings, these elements apparently derive from the marginal zone of the follicles. Here numerous mesenchymal, undifferentiated cells are found proliferating, especially during the infectious process, and apparently producing the monocytes, which emigrate and fall into the lumen of the perifollicular sinuses.

Therefore, a picture similar to that observed in the lymph-nodes is found in the spleen. In fact, at the periphery of the lymph-node follicles, undifferentiated or germinal cells can be seen which are equally considered as a source of monocytes (*1*).

There are, then, evidences that two types of cells, with different morphological characteristics, derive from the lymph follicles or Malpighian corpuscles of the spleen, viz., cells that are typical lymphocytes, which come from the germinal centers, and the elements we have described as monocytes, which apparently originate from the marginal zone of the follicles.

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Ultraspectrophotometric Studies in Extracts of Normal and Tumor Tissue of Human Origin

MACHTELD E. SANO and MONA SPIEGEL-ADOLF

Temple University Medical School and Hospital, Philadelphia

Histologic properties frequently have a chemical correlate traceable also after cell destruction. It seems highly probable that the cell characteristics of malignant tumors should become manifest in their extracts as an increase of the nucleic acids or their cleavage products. Claude and Rothen (*1*), Thomas (*3*), and Stowell (*2*) suggest that nucleic acids or their protein compounds may be part of the active principle of tumor formation. Our studies are based on ultraspectrophotometric analysis of tissue extracts.

Tissues were obtained at operation in 26 cases, postmortem in 4. Fourteen specimens were from malignant tumors, one each of Hodgkins' disease and leukemia, and three from benign tumors. Seven were inflammatory lymph-nodes, one an inflammatory tumor. The tissue was finely cut, suspended in saline solution (pH 7.2–7.4), and heated ½ hour at 65°C. to inhibit enzyme action. It was centrifuged for ½ hour at 9,000 r.p.m. in an angle centrifuge. For ultraspectrophotometric studies, a DU Beckman electric quartz spectrophotometer was used. Comparison of the optical density of the solutions was made in the same quartz cell at equal degrees of dilution. Measurements were made in steps of 10 A. between 3,100 and 2,200 A.

Normal leucocytes showed a faint indication of selective absorption at 2,600 A., but the graph looks rather S-shaped within a wave-length range between 2,800 and 2,500 A. Normal liver and endometrium are characterized by selective absorption with a peak at 2,650 A. Seven inflammatory lymph-nodes gave uniformly selective absorption with a peak in 6 cases at 2,500 A. An inflammatory tumor of the breast had an S-shaped absorption graph. Nine carcinomas showed selective absorption at 2,600 A.; a scirrhous tumor had an S-shaped graph. The height of the peak seemed in certain relation to the number of cells in the neoplastic tissues. One case, a medulloblastoma, was distinguished by two peaks at 2,550 and 2,700 A. Three lymphosarcomas and one Hodgkin's case gave similar absorption graphs with peaks at 2,600 A. Of the lymphosarcomas, one was atypical but was so clinically and histologically, diagnosis being based on tissue culture. The absorption peak in a case of lymphatic leukemia (chronic) was 2,500 A. Of the three benign tumors, two showed no selective absorption, one a low peak at 2,550 A.

Tentative suggestions can be offered regarding the selective absorption peak at 2,650 A. in normal liver and endometrium, 2,600 A. in carcinoma, and 2,500 A. in the inflammatory nodes.

From a diagnostic point of view, these differences in various pathological conditions seem of interest. The underlying causes will be the subject of further research.

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Inactivation of Staphylocoagulase by Trypsin and Pepsin[1]

BURNHAM S. WALKER, NORWOOD K. SCHAFFER, and MATTHEW A. DEROW

Boston University School of Medicine

Staphylocoagulase is a substance, present in filtrates of cultures of pathogenic staphylococci, which has the property of coagulating plasma. Despite its name, which would imply enzymic character, little is known of either its chemical nature or its mode of action. Walston (*2*) found that the active principle was not dialyzable from crude filtrates and was present in the precipitates obtained with alcohol, dilute acetic acid, or half-saturated ammonium sulfate. He also stated that the activity is destroyed by tryptic digestion. The present report is concerned with further study of the effects of trypsin and pepsin on the activity of staphylocoagulase.

Cultures of a coagulase-positive *Staphylococcus aureus* grown for 7 days in tryptic digest medium were used as the source of coagulase. After incubation, 0.5 per cent of phenol was added as a sterilizing agent, the organisms removed by centrifugation, and the clear supernates used. Coagulase activity was titrated by a serial dilution procedure in which 0.5 ml. of each dilution was mixed with an equal amount of fresh, sterile, citrated human plasma. The titer of coagulase was considered to be the highest final dilution yielding a clot filling about half the volume of the mixture at the end of 2 hours.

TABLE 1

PROTEOLYTIC DIGESTION OF STAPHYLOCOAGULASE

Enzyme	pH of digestion	Coagulase titer		Percentage decrease in protein N
		Before digestion	After digestion	
Trypsin	8.7	128	0	77
"	8.7	128	0	74
"	8.7	128	0	81
"	8.7	64	0	80
Inactivated trypsin	8.7	64	64	0
None	8.7	128	64	0
Pepsin	2.0	64	0	70
"	2.0	128	0	64
"	2.0	128	0	72
Inactivated pepsin	2.0	64	64	0
None	2.0	128	128	0

Commercial trypsin (Pfanstiehl) and U. S. P. pepsin (Merck) were used. Five ml. of the supernate plus 20 mg. of the enzyme were incubated at the appropriate pH and 37°C. for a period of 2–2.5 hours, with occasional shaking. Controls were run for each enzyme using heat-inactivated enzyme and also with no enzyme present. Before carrying out the titration of coagulase, tryptic action was checked by heating for 5 minutes at 80°C.; peptic action, by adjusting the reaction to pH 8. The preparations were cleared by centrifugation, and the coagulase titer was then determined. The activity of the enzyme preparations was checked by measuring the protein nitrogen, *i.e.* that precipitated by 5 per cent trichloroacetic acid, before and after the incubation.

The results are shown in Table 1. There was complete destruction of coagulase activity in all experiments with active enzymes. There was a one-dilution decrease in coagulase activity in one of the controls, otherwise no decrease in the controls.

This work bears upon the question of the enzymic or non-enzymic nature of staphylocoagulase. Its extreme resistance to thermal inactivation (*1*) raises some doubt as to its being a typical enzyme. The complete destruction of coagulase activity by trypsin or pepsin indicates that it contains peptide linkages hydrolyzable by these enzymes. If coagulase activity depends upon intact protein structure, it is remarkably stable when heated, since we have been able to confirm Gengou's observations and extend them by the observation that 20 minutes at autoclave temperature (120°C.) does not abolish the coagulase activity of staphylococcal culture-supernates.

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Linkage Between the Genes for Sickle Cells and the M-N Blood Types[1]

LAURENCE H. SNYDER, HENRY RUSSELL, and EUGENIA B. GRAHAM

Department of Zoology and Entomology, The Ohio State University, Department of Medicine, Washington University School of Medicine, and *Barnes Hospital, St. Louis*

It has often been pointed out that the detection of instances of linkage in man is of importance not only in an academic sense but also in order to make more precise genetic prognoses for the occurrence of anomalies and diseases (*3, 4*) and to institute preventive measures on the basis of early recognition of preclinical signs (*2*). Various methods of analyzing family data for linkage have been formulated (*1, 3*).

We have recently been investigating the linkage relationships of the genes for sickle cells and for the various blood groups and types. To date, 33 families have been tested. Although we found no evidence against random assortment between the gene for sickle cells and the genes for the A-B blood groups and the Rh types, we did find evidence that the gene for sickle cells is linked with those for the M-N blood types.

In order that any family may furnish information on the linkage between two genes, it is necessary that at least one parent be heterozygous for both pairs of genes. The gene for

[1] Studies in Human Inheritance XXXIII.