The Mixed Agglutination Reactions in the Study of Normal and Malignant Cells

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SUMMARY

1. Two reactions which appear well suited for studying the antigenic structure of the membrane of tissue cells are described. These are the mixed agglutination reaction and the mixed antiglobulin reaction.

2. By exhaustively mapping the antigens on the membrane of normal cells, it should be possible to observe any deletion of these antigens in cells which have become malignant.

The possibility that the malignant transformation of cells may entail a change in the antigenic structure is generally accepted. This change may involve the acquisition of new antigenic substances or may be in the nature of a deletion or loss of antigenic components. If an associated antigenic change could be firmly established, then its further analysis might lead to fundamental causes and methods of reversing or countering the process.

In the same way as it is impossible to study pathological changes in tissues without a knowledge of the normal histology, so it is also somewhat forlorn to embark on a search for detailed antigenic changes in malignant cells without a full knowledge of the antigenic architecture of normal cells. It is here that we have to confess abysmal ignorance.

For many years Witebsky has championed the importance of full and comprehensive studies of the specific antigens of organs and tissues, and some of this work will be discussed in another part of the Symposium. These studies have, in recent years, received a renewed stimulus by the incrimination of auto-immunization in human disease and the renewed interest in immunological theories of cancer. The new serological methods developed in the last two decades are almost a guarantee of success for such investigations.

In an attempt to make a blueprint of the antigenic architecture of cells, one must consider:

1. **The "soluble" antigens of the cytoplasm and endoplasmic reticulum.**—Studied by complement fixation reactions and recent modifications of the precipitation reactions in agar gel, immunoelectrophoresis, and Coon’s fluorescent antibody technic.

2. **Histocompatibility antigens in the nucleus.**—At present studied by transplantation procedures and possibly by the delayed-type hypersensitivity reactions.

3. **"Fixed" antigens of the cell membrane.**—Studied by cytotoxic reactions and recent modifications of the agglutination reaction, such as the mixed agglutination reactions.

It is the object of this paper to present the theory and practice of the mixed agglutination and mixed antiglobulin reaction, in the hope that they may be of value in mapping any antigenic variation in the cell membrane which may accompany a malignant transformation.

**MIXED AGGLUTINATION REACTION**

This was originally developed to demonstrate the presence of the A and B antigens on platelets and skin epidermal cells (3, 4). The method promised to be generally applicable for the study of cells which would be unsatisfactory in ordinary agglutination tests because of their shapes or size, a propensity to aggregate spontaneously, or where only very few cells were available for examination.

The rationale of the method is illustrated diagrammatically in Chart 1. The procedure of testing is simple. Antiserum is added to the cell suspension, and after a period of incubation the cells are washed to remove the serum and uncombined antibody. A suspension of indicator red cells, possessing the antigen in question, is then added, and the two cell types brought into contact by gentle centrifugation. The deposited cells are
then transferred to a 3 X 1-inch microscope glass slide and, with a coverslip overlaid, examined under the phase microscope. The appearance of a negative and positive reaction is shown in Figs. 1A and B.

The reaction can be used for any membrane antigen as long as a suitable indicator cell, possessing the antigen in question, is available. This, of course, need not necessarily be a red cell.

**ISO-ANTIGENS ON TISSUE CELLS**

The A and B blood group antigens are iso-antigens so far as the human species is concerned. Besides leukocytes (9) and platelets, a wide variety of other body cells have been examined for the A antigen (11). This latter study, however, was only a cursory investigation and was undertaken mainly to show the adaptability of the method.

It was fully realized that each cell type of the body needed a careful study of its own, because, with the A antigen especially, there is the question of its mosaicism of sub-specificities and the influence of the soluble form of this antigen in the tissue fluids. Some of the intricacies which may be encountered have been shown up in an investigation by Swinburne, Frank, and Coombs (15) on the expression of the A antigen on human buccal epithelial cells. With these cells there is a marked difference in the expression of the antigen as between A1 and A2 non-secretor persons. Also, the antigen may be firmly adsorbed onto negatively reacting cells from A-secretor saliva, and rabbit anti-A sera were shown to react differently from human so-called "immune" anti-A sera.

Concerning other human iso-antigens, it is only leukocytes that have been examined in any detail. An MN-type antigen has been shown as well as Tja. So far, we have failed to show the Rh antigens, despite claims to the contrary by persons using the antiglobulin consumption test. With such a rich and well documented body of knowledge on the iso-antigens of human erythrocytes, human tissue cells should be exhaustively studied for these same antigens. Cells in culture should be particularly useful for this purpose.

With the rapidly expanding knowledge of the blood group antigens of animals, similar studies can be prosecuted on animal tissue cells. We are, ourselves, most anxious to use the mixed agglutination technic to study the Hr antigens on mouse tissue cells.

"SPECIES ANTIGENS" ON TISSUE CELLS

By "species antigens" is meant those antigens which go to characterize the species and which, unlike the iso-antigens, are present on the cells of all individuals of the species. The membrane of a cell must be constructed with both iso-antigens and "species antigens."

These "species antigens" on tissue cells, or at least some of them, can be brought into reaction by mixed agglutination by virtue of the fact that these antigens, like some of the iso-antigens, are also to be found on both tissue cells and red cells of the same species. Demonstration of these "species antigens" on tissue cells by mixed agglutination has found an immediate practical importance in the serological identification of cells.
growing in culture (5). The rationale of the method with the use of diagnostically specific red cell antisera is illustrated diagrammatically in Chart 2. With this reaction it has been shown that "L" cells growing in culture still react strongly as mouse cells, while "ERK" cells, reported to be of rabbit origin, behave as human cells (6). It has been possible to differentiate rat and mouse fibroblasts by producing the rat red cell antiserum in mice and the mouse red cell antiserum in rats.

From the point of view of the present discussion, it is clear that although they are "species antigens" which are involved in these reactions, antigens whose specificity may be dependent on a carbohydrate moiety and which are found apparently randomly distributed throughout the various species of the animal kingdom. The best known example is the Fossman antigen.

As a membrane antigen, it may also be demonstrated on individual cells by mixed agglutination, with either sheep, dog, or cat erythrocytes (all carrying the Fossman antigen) as the indicator cell (10). In a study of this antigen on cells growing in culture (7), it was shown that cells from Fossman-positive animals could be clearly distinguished from those of Fossman-negative ani-

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**CHART 2.—Diagrammatic illustration of the rationale underlying the application of the mixed agglutination reaction for testing the “species antigens” on tissue cells.**

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we have no idea of the number concerned or their individual properties. An analysis and characterization of the individual reacting systems are essential. The antigens enumerated will give further markers which may be of value in searching out any antigenic change during malignant transformation. It is very fortunate that these "species antigens" on tissue cells have found importance in the serological identification of cells in culture as this, in itself, is likely to ensure that they receive adequate attention and investigation.

**Heterophile Antigens on Tissue Cells**

Besides "species antigens" and iso-antigens, immunologists classify another group of antigens under the name heterophile antigens. These are normals by tests with anti-Fossman sera. It was also shown that cells lacking the antigen could acquire the antigen by culture in the presence of serum from a positive animal.

There are many indications that there is not one Fossman antigen but rather a whole series of antigens or mosaic of subspecificities. If this be so, then, as in the case of the "species antigens," when these are analyzed and separated, many more antigenic markers will be available for a comparison of the normal and malignant cell.

**Mixed Antiglobulin Reaction**

So far, we have been discussing the mixed agglutination reaction, which is essentially a method for demonstrating antigens on tissue cells, and
more specifically antigens which have been characterized to a certain extent and which are also available on a suitable indicator cell.

The mixed antiglobulin reaction, on the other hand, is a method for demonstrating antibody to tissue cells. The antibody may be a hetero-, iso- or auto-antibody, and it is not necessary to have any knowledge of the membrane antigen involved. The principle of the reaction is shown diagrammatically in Chart 3. For details of the technic the reader is referred to Chambers, Coombs, Gurner, and Dausset (2). The method was originally introduced for demonstrating platelet and leukocyte iso-antibodies in human sera, and, as yet, very little work has been done with tissue cells. In the action is the tendency of certain cell preparations to absorb globulin non-specifically, a circumstance which makes the use of the reaction impossible.

**STUDIES SPECIFICALLY DIRECTED TO MALIGNANT CELLS**

There is a large literature on attempts to demonstrate and isolate cancer-specific antigens, and this will probably be critically reviewed by other contributors to this Symposium. Suggestive leads from the older literature can now be followed up and extended, by the more recent methods of precipitation in agar gel and immuno-electrophoresis.

According to recent theories on carcinogenesis...
likely was the antigen to be absent or masked. Further studies along these lines should be most interesting.

As yet, we have not ourselves studied malignant cells freshly isolated from the body. What we have made objects of testing are HeLa cells, and here the “species” antigens appear to be well developed and of the “blood cell iso-antigens,” H, and MN-type antigen and Tja are present (13). These cells, as expected, do not possess the Fossman antigen but will acquire it if grown in serum from a Fossman-positive animal. As mentioned in the previous section, “leukocyte iso-antigens” are also to be found on the membrane of HeLa cells (see Figs. 2 A and B), although whether they contain their full quota cannot be told with our present poor knowledge of this group of antigens.

“L” cells also react powerfully with antisera to the “species antigens” of mouse red cells, but they appear to have dropped the Fossman antigen (7), which is present on recently isolated mouse tissue cells.

To conclude this section, it would seem that the mixed agglutination and mixed antiglobulin reactions could profitably be used, along with the other existing immunological methods, to map, as completely as possible, the antigenic structure of normal cells of an inbred animal line. A similar examination of the cells of a spontaneous or induced tumor might well afford further evidence on this question of antigen loss during malignant transformation.

REFERENCES

FIGS. 1-2.—Phase microscopy, X10 eyepiece, X40 objective.

Fig. 1.—Examination of human buccal epithelial cells for the “A” antigen by mixed agglutination.
A: negative reaction; B: positive reaction.

Fig. 2.—Mixed antiglobulin reaction on HeLa cells. (Taken from Chambers et al., 1959.)
A: positive result with leukocyte iso-antiserum, showing mixed aggregates of sensitized HeLa cells and the sensitized red-cell indicator system; B: negative result with normal human serum. No mixed aggregates. Separate clumping of the sensitized red-cell indicator system.
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