Opinions are divided as to how far fluorescent-labeled antibodies (F.A.) lend themselves to the elucidation of the immunological conditions present with malignant tumors. It is, therefore, of interest to discuss the results achieved up to the present, especially because the specificity of the method has recently caused much discussion.

Only a few investigators have used fluorescent antibodies to demonstrate virus antigens in tumors. In Shope papilloma, by use of fluorescent immune sera, a large amount of intranuclear virus antigen was demonstrated in the keratohyaline layer in wild rabbits, whereas only negligible amounts were found in the tame rabbits (20). Antigen could not be demonstrated in the proliferating epithelial cells, probably owing to lack of immune activity of the virus nucleoprotein.

In the Rous sarcoma Mellors (15) and Malmgren and co-workers (12) found mainly cytoplasmic, in part diffuse, but often spherical fluorescent bodies, and they were able to show a certain parallelism between the amount of the tumor-producing virus injected and the fluorescence demonstrated, regardless of the size of the tumor—a finding which is supported by electron microscopic and biological investigations.

In biopsies from patients given inoculations of West Nile virus, in a therapeutic trial, the virus was demonstrated by means of the F.A. method in cancer cells and leukocytes, but it was not present in the stroma (23).

Most investigators have used fluorescent antibodies along with other serological methods to evaluate the antigenic properties of malignant cells compared with homologous normal cells.

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the lack of fluorescence. In other words, lack of fluorescence with protein conjugates is, in their opinion, a criterion of malignancy, and they recommend it as a practical diagnostic aid with different malignant tumors. They have found the same contrast between benign and malignant proliferation with Shope papilloma, without its being possible to demonstrate intracellular fluorescence even with the help of conjugated specific immune sera (10). Malignant cells in tissue culture give no fluorescence, and normal cells under similar circumstances gradually lose their ability to show fluorescence—indicating, according to Louis, a preneoplastic change (11). On the other hand, Pressman and his co-workers hold that they can demonstrate immunologically specific fluorescence in malignant cells in tissue culture treated with anti-HeLa conjugates, but they also showed wide cross-reactions with normal tissues (3).

Later workers have not supported the Australian groups' opinion that the lack of fluorescence in malignant tumors is due exclusively to a physico-chemical reaction. Nairn et al. (19) have extended Weiler's investigations, in that they have treated, in addition to hepatoma and renal carcinoma, many human skin cancers with conjugated organ-specific sera. In their experience the fluorescence due to the organ-specific antigen disappears after absorption of these sera with homogenate, whereas absorption with tumor homogenate does not completely remove this fluorescence, indicating that this antigen is lacking or at any rate greatly reduced in tumor cells. Thus, their results support Weiler's immunological contention.

The latter authors have investigated the difference in fluorescence between normal and tumor tissue treated with nonimmune conjugates (18). Most, but not all, of the carcinomas show less fluorescence than do the corresponding normal tissues. The difference is, however, as a rule very slight and has nothing in common with what one sees with the specific immune staining. The mucous membrane of the colon is an exception, since in most cases this fluoresces to a much greater extent than does the corresponding carcinoma. This lack of uniformity in the results detracts from the usefulness of the technic in the diagnosis of malignancy. Hiramoto and his co-workers also retain their belief in the immunological specificity of the fluorescence (4), and they attribute the lack of fluorescence in some rhabdomyosarcomas, compared with that in normal striated muscle, to the disappearance of the myosin in the antigenic structure of these tumors (5).

Pressman and his co-workers produced antiepidermis and antimelanoma conjugates to study the histogenesis of melanoma (6). They found that there were common antigenic properties between these tumors and neurolemma, and thus they consider that they can support Masson's theory that melanomas are of neuroectodermal origin (5).

In the works quoted above hetero-immune sera were used. G. Möller (16) has demonstrated isoantigens in many inbred strains of mice and in tumor tissues from mice. The so-called ring-reaction—i.e., a bright fluorescence outlining the cell surface—was stated to be specific. The demonstration of isoantigens was carried out mainly on isolated cells, since, in some antigen systems, unspecific reactions occurred in histological sections.

Thus, in the view of the majority of investigators the F.A. technic can elucidate the immunological conditions present in neoplastic tissue. The fluorescence that occurs with the use of nonimmune conjugates in most normal tissues and benign tumors and the loss of fluorescence in the corresponding malignant tumors, claimed by the Australian group, is, in our experience also, easy to distinguish from the immunological specific reactions—not only by the difference in the localization and intensity of the fluorescence, but also with help of control methods (14, 17) that should be used in the evaluation of whether the fluorescence observed is immunologically specific.

By means of the fluorescent antibodies it has been possible to transfer the technic of immunological cancer research to cellular structures. By its use in this area it has proved possible not only to confirm earlier results obtained by serological in vitro methods or from tissue culture, but also to produce results in which a correlation between the immunological reactions and the histological structure was of significance.

Coons's indirect method, which is also called the "sandwich-method," has been used by most tumor investigators, not only because it gives 4-12 times stronger fluorescence than the direct method (21), but also because with this method any absorption of the sera that may be necessary can, with advantage, be carried out on the unconjugated sera that are used in the first stage of the reaction.

There is extremely little information in the literature concerning the sensitivity of the F.A. method compared with other methods used in immunological cancer research. In patients who had received therapeutic virus injections and had formed antibodies against the virus, the fluorescent antibody technic gives more positive results than virus isolation (23).

Compared with the radioactive tracer technic (21), the F.A. method has the advantage of better localization of the antibody on a cellular basis. On the other hand, the minimum amount of antibody
that can be demonstrated is lower with the isotope technic.

The greatest drawback to the F.A. method is the occurrence of unspecific staining reactions—i.e., fluorescence that is not due to an immunological reaction between antigen and the corresponding marked antibody. Neutrophils, eosinophils, histiocytes, and some other cells will often give a nonspecific reaction; but other tissues may also be involved in this type of fluorescence, which can be very troublesome to the interpretation of the results. The removal of the unbound fluorescent material from the conjugate by means of prolonged dialysis or gel-filtration (sephadex) does not prevent these reactions. Many consider routine absorption of the conjugates with organ powder to be unsatisfactory, since such absorption will probably not hinder unspecific reactions of a physico-chemical type (13). Several workers have recently recommended fractionation of the conjugates by chromatography on DEAE-cellulose as an effective method of avoiding unspecific fluorescence (1, 2, 22). Goldstein’s work (2), in particular, is well documented and presents some surprising conclusions. In his experiment, in which conjugated sheep-antirabbit globulins were applied to spleen imprints, definite specific reactions occurred only in those conjugates in which the amount of fluorescein used for the preparation of conjugates did not exceed 6–8 mg/gm protein (50 mg/gm protein is usually used), assuming that the protein concentration in the final conjugate is about 1 mg/ml. A critical evaluation of the application of the chromatographic method to the fluorescent antibody technic is not available at present. Our experience seems to support the view that chromatographic treatment of conjugates forms a very satisfactory method of removing unspecific fluorescence.

On the other hand, in our experiments with rabbit-antihorse globulin it was found that protein concentrations of about 1 mg/ml conjugate were insufficient to produce a recordable fluorescence.

REFERENCES

Fluorescent Antibodies in Cancer Research: A Review

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