Self–nonself concept for cancer and diseases previously known as “autoimmune” diseases

(illlegitimate transferases/plasma exchange)

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Contributed by Philip Levine, August 16, 1978

ABSTRACT The illegitimate glycosphingolipid antigens of the P blood group system and of the Forssman (Fs) tissue antigen in adenocarcinoma which are foreign to the host suggest the self–nonself concept which applies also to numerous other diseases such as rheumatoid arthritis, lupus, glomerulonephritis, and idiopathic acute hemolytic anemia. In the presence of the glycosphingolipid antigens such as ABO, P, and Fs, the normal serum of the homozygote recessive precursor contains antibodies for the missing antigen(s). The expected antibody to the Fs antigen was present in about 75% of normal men and women. In cancer sera, the incidence of anti-Fs was decreased to about 35–40%. On testing the normal population anti-Fs was present in 90% of the sera in the youngest group, and this value gradually diminished in the older groups; the incidence of the antibody in the 70-year age group was to about 60%. The rate of loss of anti-Fs with increasing years appears to parallel the gradual loss of anti-A and anti-B isoagglutinins. This phenomenon may be associated with the gradual diminution of protein synthesis with aging or the continuous accumulation of soluble immune complexes in the serum, or both. It is suggested that the self–nonself concept is also the basis for the pathogenesis of rheumatoid arthritis, lupus erythematosus, idiopathic acute hemolytic anemia, and numerous other conditions classified as “autoimmune” diseases. Some of these diseases are induced by viruses or drugs or both. When a virus or drug attaches itself to the membrane of a tissue cell, the self is converted to nonself which, in rheumatoid arthritis, alters its self Ig to nonself Ig.

In biochemical studies of surgically removed adenocarcinoma of the stomach and colon, Hakomori et al. (1) demonstrated that in 16 of 21 cases the normal mucosa contained the glycosphingolipid tetrasaccharide identified as globoside. The terminal sugar in globoside is β-N-acetylgalactosamine (β-GalNAc) but the adjacent neoplasms contained an additional sugar, α-GalNAc. The latter is the terminal sugar common to both the Forssman (Fs) and blood group A antigens and this is the structural basis for the cross-activity of anti-Fs with group A erythrocytes. The Fs antigen is derived from globoside whereas the group A antigen is derived from paragloboside. Globoside, which is fsfs, is precursor to Fs. Apparently, the adjacent cancer in the 16 cases produced an illegitimate transference that attached a bastard terminal α-GalNAc to globoside (1).

Although globoside extracted from the normal mucosa failed to inhibit the action of anti-Fs—i.e., hemolysis of sheep erythrocytes (SRBC) in the presence of complement—the adjacent cancer tissue did inhibit the specific hemolysis of the standard anti-Fs as well as anti-Fs produced by injection of Fs-containing liposomes derived from the neoplasms (1). These reactivities for both the 16 cases with the fsfs → Fs conversion in the adjacent cancer and the 5 cases in which the Fs antigen was found in the normal colon tissue have been summarized in tabular form (2).

The ffs-Fs polymorphic isontigen is limited to tissue cells and thus differs from the pp–P1 isontgens reported by Levine et al. (3) in 1951 in which the P antigen is present on erythrocytes and presumably also on tissue cells (4). Thus, specific binding of anti-Fs by the illegitimate Fs antigen in adenocarcinoma is quite analogous to the specific absorption of the pp patient’s high-titered anti-P1 (of the anti-P1,P2p complex) by her own lyophilized gastric cancer tissue characterized by the illegitimate pentasaccharide P1 Fs antigen (3).

Therefore the precedent for the fsfs → Fs conversion has been established, especially so after its 1974 updating to incorporate the biochemical data of Marcus et al. (5, 6) on the series of steps involved in the synthesis of the several antigens of the P system ending in the final P1 pentasaccharide. It is assumed that the rare P antigen, apparently the precursor to the final P1 antigen, is a sialylparagloboside (6). By some unknown mechanism the p precursor is converted in the neoplasms to its final product with its illegitimate P1 antigen consisting of a paragloboside chain—a tetrasaccharide—with its additional terminal α,1-4-galactose. At the same time, the branched sialic acid of the P antigen was lost, probably by the action of a sialidase. Additional biochemical support for these assumptions is still to be provided.

Both ABO and P antigens are derived from paragloboside whereas the Fs antigen is synthesized from globoside. The precursor substances HH (for group O) and pp are characterized by antigens on both the erythrocytes and their tissue cells, and the serum contains antibodies specific for the missing antigens. In this and other isospecific systems, the precursor substance is believed to be recessive and homozygous whereas the final product is obviously dominant (2). The presence of anti-P1, anti-P2, and anti-P4 in the serum of pp individuals is quite analogous to the presence of anti-A and anti-B in the sera of group O individuals. Accordingly, it was assumed that the serum of individuals whose normal colon or gastric mucosa contained the globoside precursor should contain an antibody specific for Fs—i.e., specific for its missing fifth and terminal sugar, α,1-3-GalNAc.

EXPERIMENTAL

The test for anti-Fs was carried out with heat-inactivated serum diluted 1:8 and guinea pig complement 1:30 diluted to which a 2% suspension of washed SRBC was added: 2 drops each of the three reagents were mixed in small test tubes (10 × 75 mm) and incubated for 1 hr with occasional shaking at 37°C (water bath). Clear-cut results were obtained by recording the degree of hemolysis. If hemolysis was complete, no SRBC remained as a sediment when the tubes were centrifuged at about 500 × g for 2 min. Lesser degrees of hemolysis correlated with the quantity of the sedimented SRBC.

Acknowledgments

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Abbreviations: Fs, Forssman antigen; GalNAc, N-acetylgalactosamine; ICs, immune complexes; SRBC, sheep erythrocytes.
As shown below, this was established when tests with numerous serum samples from a normal population showed that more than 75% of the sera contained the predicted antibody. The antibodies in both HH (group O) and pp individuals contain IgG as well as IgM molecules (Table 1). IgG molecules were demonstrated biochemically in sera of HH individuals and in sera of pp individuals (8). Only IgG molecules cross the placenta to induce ABO hemolytic disease of the newborn and some abortions in group O women and ABO-like hemolytic disease of the newborn as well as antibody-induced abortions in pp women. The data in Table 1 also indicate the association of adenocarcinoma in the 1951 pp patient with its pp → F₁ conversion in the gastric adenocarcinoma (2, 3) and in the larger group of 16 cases reported by Hakomori et al. (1) which showed the fsfs → Fs conversion.

The incidence of anti-Fs in sera of a normal population was 79%, in contrast to 32–39% in sera from patients with cancer (mainly adenocarcinoma (Table 2)). Tests on freshly drawn specimens from cancer patients also showed the greatly reduced incidence of anti-Fs. Both the human and the experimental anti-Fs are specifically absorbed by Fs containing guinea pig kidney tissue and SRBC but not by beef tissue (2). Anti-Fs is not absorbed by group A erythrocytes which lack the β₁-3-GalNAc of globoside.

Osborne and DeFrancis in 1962 and 1963 (9, 10) studied the incidence of the ABO erythrocyte antigens in patients suffering from cancer of the ovary or salivary glands or from metastasis in general. They confirmed the 1953 report by Aird et al. (11) that group A individuals are somewhat more susceptible to gastric cancer. Although statistically significant, this association was quite weak and was either not confirmed or completely denied by others (12). Osborne and DeFrancis tested only the erythrocytes for the ABO antigens but preserved the sera in the frozen state. When a test sample of 55 of about 5000 serum specimens collected in 1960–1962 from patients at Memorial Hospital was recently tested for anti-Fs, only 32% contained the hemolysin (Table 2).

The most likely explanation for the conversion of 79% hemolysins in a normal population to 40% in patients suffering from adenocarcinoma is based on the following: (i) the presence of illegitimate antigens in the neoplasm which are foreign to the host; (ii) the identification of the biochemical nature of the antigens as glycosphingolipid (1, 5, 13); and (iii) the presence of natural antibodies in the normal serum specific for the absent antigens as described for the 1951 pp patient and the demonstration of natural anti-Fs in normal sera of fsfs individuals (Table 2).

Thus, the adenocarcinoma is specifically coated with its own

<table>
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<th>Table 1. Precursors and final products</th>
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<tr>
<td><strong>Precursor Antigen</strong></td>
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<td>HH</td>
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All precursors are homogenous and recessive to their final products, as indicated above for pp and fsfs. HH should be relabeled as hh, but this term was assigned to the ABHnull type previously identified as the Bombay type (7).

* a, Fucosylparagloboside or fucosyltetrasaccharide; b, ceramide lactoside accumulates; sialylparagloboside inhibits action of the rare and only available "anti p" by a P₁ individual, presumably of phenotype P₁P₁ (6); c, globoside.

** HDN, hemolytic disease of the newborn; ab, antibody-induced abortion; ca, adenocarcinoma.


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<th>Table 2. Anti-Fs in heat-inactivated sera in presence of guinea pig complement 1:30</th>
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<tr>
<td><strong>No. tested</strong></td>
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<tr>
<td>Normal*</td>
</tr>
<tr>
<td>Cancer†</td>
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<td>Cancer‡</td>
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<td>Total, cancer</td>
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Sera were diluted 1:8; complement was diluted 1:30.

* These sera from freshly drawn blood were obtained from blood donors at the New York Blood Center and employees at Memorial Sloan-Kettering Institute and Ortho Research Foundations.
† This series represents a sample of 55 from about 2000 sera from patients at Memorial Hospital stored at −30°C in the frozen state by Osborne and DeFrancis (8, 10).
‡ These sera were derived from patients with adenocarcinoma at Memorial Hospital and were stored frozen at −30°C.

specific antibody and it is assumed that these antibody-coated malignant cells take up C1q to form immune complexes (ICs) of heavy (20–22 S) units, which constitute the lesion on the mucous membrane. The serum contains ICs of smaller size that also bind C1q, the first component, and thus prevent it from participating in the hemolytic process of anti-Fs activity. Soluble ICs in sera of patients with several varieties of cancer as chronic lymphocytic leukemia were described recently by Day et al. (14), Teshima et al. (15), and Theofilopoulos et al. (16).

Confirmation of the hypothesis described above was achieved unexpectedly when the incidence of anti-Fs in the normal population was analyzed by age groups (Fig. 1). The highest incidence, >90% (200 of 221 in the youngest group), is to be contrasted with the value, 61%, for the 50–80 year group (62 of 101 with anti-Fs). These values can perhaps be related to the accumulated effect of immune responses to infections and infestations with increasing age. An almost parallel gradual diminution of anti-A and anti-B with increasing age was reported by Thomsen and Kettel (17) in 1929. Possibly both age-related findings are an expression of decreasing protein (enzymes, immunoglobulin) synthesis associated with the natural aging process.

One may suggest that, in cancer and other diseases associated with ICs, the total effect of soluble ICs in the serum is an exaggeration of the normal phenomenon of aging in the older group of normal individuals. Thus, the still lower incidence of anti-Fs in sera of the cancer group, in contrast to the older normal population, may be associated with the increasing growth of the neoplastic lesion characterized by formation of high molecular weight ICs. Although large quantities of complement are bound to the fixed lesion, enough may be available to release to the serum small quantities of soluble complexes resulting from the solubilization by the cascade of complement-derived enzymes acting on the lesion (18), particularly after therapeutic plasma exchange using young donors. As discussed below, such a procedure may be responsible for favorable therapeutic effects from plasma exchange (unpublished data).

The test for the presence or absence of anti-Fs is a rapid screening procedure which yields information on the presence of soluble ICs in any given serum. However, this test should be followed by more sensitive tests specific for the binding or deviation of Clq, the radioimmunoassay by the Raji lymphoblastic cell, or other numerous tests described in the literature. In preliminary tests using EDTA to inactivate total complement in test sera, it has been shown (H. Kitamura, P. Levine, V. Mikes; R. Egeli, R. A. Good, and N. K. Day, unpublished data) that the
procedure will detect total anti-Fs whereas the rapid screening test with heat-inactivated sera detects soluble ICs in addition to anti-Fs. The higher incidence of anti-Fs in the younger normal group as compared to the older group was confirmed.

DISCUSSION

The recent developments leading to a fuller appreciation of some fundamental aspects of adenocarcinoma resulted from: (i) techniques for solubilization of the erythrocyte membrane; (ii) the stepwise biosynthesis of the several antigens of the ABO, P, and Fs glycosphingolipids; and (iii) the biochemical analysis of blood group genetic markers on erythrocyte and tissue cell membranes.

The globoside (fFs-fs-Fs) glycosphingolipid antigens form a polymorphic system with globoside as the precursor for its final Fs product appearing on the mucus of the gastrointestinal tract. The Fs antigen is present in SRBC but cannot be demonstrated in human erythrocytes. The significant findings of Hakomori et al. (1) led to the discovery of anti-Fs as a natural antibody in normal human sera which hemolyzes SRBC in the presence of complement. Anti-Fs is age-related in a normal population: the highest incidence (90%) found in the younger age group gradually diminishes so that only 59% is observed in the age groups 60–80 years (36 hemolyzers among the 61 individuals). Further studies with more defined methods are required to detect soluble ICs in normal sera and to confirm that formation of ICs increases with the natural aging process.

It is assumed that these findings for a normal population and the still lower incidence (38–40%) of anti-Fs in cancer sera are associated with ICs resulting from two circumstances: (i) the characteristic presence of normal antibodies in the serum for the absent glycosphingolipid antigens (Table 1); and (ii) the presence in the neoplasm of the final product—i.e., the illegitimate antigen that is genetically foreign to the host (1–3).

Thus, the situation is ripe for the formation of soluble ICs in the serum which, by means of one or another mechanism, such as that discussed by Cochrane (19), find their way into selected target sites to induce lesions of high molecular weights (20–22 S). In cases of malignancy, the most frequent sites are colon, endometrium, and stomach as shown in one of the remarkable cancer families described by Lynch (20) with a 13.4% incidence of several varieties of cancer among 842 members.

The ABO system has thus far not been directly associated with cancer, and the observations by Aird et al. (11) and Osborne and DeFrancis (9, 10) on the greater susceptibility of group A individuals to adenocarcinoma can be related to the finding that the Fs and blood group A antigens have the identical α-GalNAc in their thermal positions. As mentioned above, the A antigen is derived from paragloboside but Fs antigen is derived from its precursor, globoside. Whereas the specificity of anti-A or anti-P1 is attributable to the terminal nonreducing sugars—α-GalNAc or galactose, respectively—the specificity of anti-Fs is directed to two consecutive molecules of GalNAc, the one β-attached followed by the terminal α-attached (21).

Of considerable significance is the presence, in the antibodies of the pp patient, of IgG (as well as IgM) molecules (7). Reasoning by analogy also with the character of the antibodies in the ABO system, it was assumed that anti-Fs would not contain IgC molecules. The presence of IgG molecules is the basis for antibody-induced hemolytic disease of the newborn and abortions. These pathologic conditions, however, are not associated with anti-B of group A or with anti-A of group B sera because the lower molecular weight IgG molecules have not been found in the sera. IgG molecules in the sera of group O and pp individuals are associated with the presence of more than one antibody. These circumstances are of considerable im-

![Figure 1](https://example.com/figure1.png)

**Fig. 1.** Incidence of anti-Fs in normal population, by age groups. ■, Blood group O, anti-A; □, blood group O, anti-B; ●, blood group B, anti-A; ○, blood group A, anti-B; ▲, % normal hemolytic Forssman.
portance because there is reason to believe that the IgG quality of high-titered anti-P antibody in the patient of Levine et al. (3) may be related in part to the absence of metastasis and her 22-year postsurgical survival (she died at age 88 from natural causes). A detailed explanation for this patient's long survival, based on a two-step humoral immunity followed by cellular immunity, will be published elsewhere (P. Levine and R. A. Good).

Consequently, the outlook for specific immunotherapy in the globoside-Fs conversion type of malignancy may not be favorable because only high-affinity IgG molecules, which may be induced by immunization with small quantities of the incompatible antigen, can react with the specific genetic markers on the membrane of the malignant tissue cell.

The lower incidence of anti-Fs in the older population and the still lower incidence in cancer patients are probably associated with the presence of ICs in their serum. The first clear demonstration of ICs in cancer was by Levine et al. (3) but this association was not made clear until 1974 when the biochemical findings on the P antigens were described by Marcus (5) and his coworkers (6). The difficulty in the further analysis of these findings lies in the fact that these studies are based on the demonstration of ICs in the serum of cancer patients or the older normal population that are necessarily of the soluble variety and of low molecular weight. It will be important to determine the size, in terms of $S$ units, in sera of cancer patients and to compare them to ICs in the older normal population as well as in the cancer tissue itself.

Without taking into account the age of the individuals in the normal control series, findings in any of the diseases characterized by high values of ICs (C3 or binding of the Raji cell assay) are subject to errors in interpretation. Thus, in a recent study by Tachovsky et al. (22) on ICs in multiple sclerosis by the Raji cell assay, 33 of 67 patients showed high values but 4 of 27 normal controls showed values higher than in some of the patients. Is not one then justified in assuming either that these four normal individuals were of the older group or, if they are of the same age as some of the patients, will not these individuals soon become victims of multiple sclerosis or any one of the diseases characterized by ICs? It is now obvious that in future studies the age of all individuals in both the normal control group and the patients should be considered as essential information required for meaningful interpretation of the data.

SELF-NONSELF CONCEPT OF "AUTOIMMUNE" DISEASE

Immune complexes are found not only in cancer sera but also in sera of rheumatoid arthritis, lupus erythematosus, glomerulonephritis, and many other disease states referred to in the literature as "autoimmune" diseases. ICs in such sera are frequently associated with the main lesion in the several diseased states characterized by deposits of high molecular weight material in selected tissues such as the mucous membranes in adenocarcinoma, joint tissues in rheumatoid arthritis, and the widespread lesions in lupus erythematosus and on glomerular membranes resulting in glomerulonephritis.

It appears that the self–nonself concept serves as a unifying principle covering the wide variety of pathologic conditions mentioned above as well as the lower incidence of anti-Fs in the older normal population. As formulated by Burnet (23) the body fails to produce antibodies to its own normal tissues. In each of the conditions mentioned above, there are environmental factors that convert "self" to "nonself". In cancer there is a nonself antigen—a terminal extra sugar determinant produced by a bastard transferase, genetically foreign to the host, which attaches a specific sugar to the precursor as the acceptor molecule (1–3). For both rheumatoid arthritis and lupus erythematosus, it has been suggested (24, 25) that viral or other agents such as drugs may alter the self IgG molecule or the self DNA or its nucleotides to the nonself variety with subsequent recognition leading to an immune response and formation of ICs that are eventually deposited in tissue sites specific for the disease.

Very likely, autoimmune hemolytic anemia with a positive direct Coombs test should be included as one of the diseases characterized by the self–nonself concept. If this condition follows a viral infection or the administration of specific drugs, these do not attach themselves to terminal low molecular weight haptenic groups of the ABO, P, or MN sites. Viruses or drugs bind to the erythrocyte membrane itself which consists of a large molecular weight lipoprotein (26) shown to possess Rh activity (27). This evidence is derived from studies of the rare Rhnull individual who has a fully compensated hemolytic anemia resulting from a leaky membrane and associated with abnormally shaped stomatocytic erythrocytes. With attachment to the Rh membrane, the self is converted to nonself, with the usual sequence of an immune response and production of antibodies directed to antigens of the Rh system (mainly to antigen e). The classical example was described by Weiner et al. (28) in a patient with epidermolysis whose erythrocytes gave a positive direct antiglobulin test.

In the literature, all diseases associated with ICs have been classified as "autoimmune." If we accept the self–nonself concept as described above, then there is no further need to retain the highly ambiguous term "autoimmune." Aside from specific lesions in the joints in rheumatoid arthritis, other immunological reactions may be demonstrable in the serum resulting from the immune response to altered (nonself) IgG. This is probably the basis for the term "anti-antibody" (29).

Numerous papers have appeared recently dealing with plasma exchange as a therapeutic measure to remove ICs in diseases such as cancer, systemic lupus erythematosus, Goodpasture syndrome, melanoma, hemolytic shock in dengue fever, malarial nephritis, extravascular symptoms of subacute bacteria endocarditis, prodromal symptoms of bacterial endocarditis, multiple sclerosis, and conditions previously not intimately associated with ICs such as hypercholesteremia and Rh-negative mothers with histories of still births. Is it not likely that more favorable results would ensue if younger donors were the source of the plasma, to supply larger quantities of IgG and complement which, with antigen, form ICs in the serum and particularly in the tissues? Miller and Nussenzweig (18) emphasized, in their experimental model, the role of the cascade of complement enzymes which tend to solubilize the large units of antigen–antibody aggregates deposited in the lesion into smaller units.

The author acknowledges the technical assistance of Mario Celano, Daniel Sujansky, and Sa An Hu. He thanks the New York Blood Center, Ortho Research Foundation, and Memorial Sloan-Kettering Institute for making available specimens of a normal population of donors and employees. He is grateful to Drs. Robert A. Good, Kenneth Lloyd, Elliot Oserman, and Gregory Siskind for discussions dealing with the contents of this paper. Supported in part by Grant 1 ROICA-20660-01 from the National Cancer Institute.

† References to papers dealing with plasma exchange in the diseases mentioned above are given in an unsigned editorial in Lancet (30) and by Schur (51).
Medical Sciences: Levine

