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Human Granulocyte Antigens: Current Status and Biological Significance (41978)

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The importance of granulocytes in fighting infectious organisms is unquestioned; people with marrow aplasia or other clinical conditions resulting in granulocytopenia frequently die from infection. Although granulocytes appear uniform, recent studies have revealed they are functionally heterogeneous (1). Granulocytes differ in their locomotion, phagocytic ability, oxidative metabolism, protein synthesis, IgG Fc receptors and expression, IgA receptors and expression, density, membrane potential responsiveness, alkaline phosphatase, and surface antigens (2). Whether these differences reflect multiple subpopulations of granulocytes or merely different stages in their maturation sequence is presently unknown. One way to investigate this question is to use antibodies to the antigens on the granulocyte surface. Are functionally different subpopulations of granulocytes recognizable by particular antigens? Do antibodies to particular antigens interfere with certain granulocyte functions? Answers to these questions will probably soon emerge (2). The purpose of this review is to describe what is presently known about granulocyte antigens.

Historically, this field began with the recognition that the transplacental passage of antibodies to granulocytes from the sera of mothers to the fetal circulation could sometimes result in isoimmune neonatal neutropenia (1, 3–7). This situation is analogous to hemolytic disease of the newborn. These maternal alloantisera were then used as reagents to identify granulocyte alloantigens (1, 3–7). Subsequently, the sera of people with certain clinical diseases were found to contain autoantibodies reactive with granulocytes, and these were also used as reagents to define granulocyte antigens (8–10). A third source of sera has been that of multitransfused patients; however, the presence of antibodies to many different specificities, such as HLA antigens, has limited the utility of this source of antisera (11, 12). Table I summarizes the use of these antisera to define granulocyte antigens. The recent development of many kinds of monoclonal antibodies which react with granulocytes has been a boon to this field, and progress in characterizing granulocyte antigens should now be rapid (Table II).

Granulocyte Antigens Classified to Date. Table I shows many of the antigens which have been detected on human granulocytes by autoantibodies or alloantibodies. These antigens can be separated into two broad categories—those shared with other cells and tissues, and those which are unique to granulocytes (1–34). In the first category, granulocytes exhibit the HLA system of antigens, erythrocyte antigens, and antigens such as 5a, 5b, and HGA 1 and 2. The group 5 and HGA antigens were originally discovered on granulocytes, and then detected on other cells (12, 19, 20). Some of the erythrocyte antigens, as shown in Table I, have only been detected through absorption-elution techniques (1). Whether the erythrocyte and HLA antigens are integral to the granulocyte membrane is presently unresolved, but antibodies to some of these shared antigens have been associated with clinical conditions (1, 25).

The other group of granulocyte antigens, those which are specific for granulocytes, are also summarized in Table I. These have been detected by autoantibodies or alloantibodies from whole serum. Because different detection techniques have been used by various investigators, a complete and nonduplicating system of antigen classification has not yet been completed. Known granulocyte-specific antigens include NA1, NA2, NB1, NC1, ND1, NE1, 9a, and the HGA-3 antigens. Although these antigens have been detected by different techniques, some have been confirmed by more than one method. It should be emphasized that the 9a, NA, NB, NC, ND, and NE antigens were described earlier

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TABLE I. HUMAN GRANULOCYTE ANTIGENS DETECTED BY AUTOANTIBODIES OR ALLOANTIBODIES FROM WHOLE SERUM

Antigen	Gene frequency	Methods[a]	Ig class of Ab	Ref.
		Granulocyte-specific antigens		
NA1	0.377 (0.32)[b]	LAT, IF, GCT	IgG, IgM	(1, 3, 4, 18, 21)
NA2	0.633 (0.68)	LAT, IF, GCT	IgG, IgM	(1, 3, 4, 21)
NB1	0.83 (0.72)	LAT, IF, GCT	IgG, IgM	(1, 3, 4, 5, 21)
NB2	0.167	LAT, IF	—	(6)
NC1	0.72 (0.80)	LAT, IF	IgG	(1, 3, 4, 7, 21)
ND1	(0.88)	LAT, IF	IgG, IgM	(4, 8, 10)
NE1	(0.12)	LAT, IF	IgG	(9, 10)
9a	0.345 (0.39)	LAT (IF-neg)	IgG	(1, 3, 4, 21)
HGA-3a-3e	0.11, 0.13, 0.08, 0.31, 0.09	GCT	IgM	(12, 13, 19)
G-A		GCT		(15)
G-B		GCT		(15)
G-C		GCT		(15)
1,2,3		GCT, LAT		(34)
Gr-1		GCT		(11)
Gr-2		GCT		(11)
1-5		Papain/GCT		(14)
		Antigens shared with other tissues		
HLA		LAT, IF, GCT		(1, 4)
ABH		GCT (IF-neg, LAT-neg)		(1, 4)
Ii, U, Jk[a], Jk[b], Kx, Ge		Absorption studies		(1)
5a	0.181	LAT	IgG	(16)
5b	0.819	LAT	IgG	(16)
HGA1,2		GCT	IgM	(12)

[a] LAT: leukocyte agglutination test; IF: immunofluorescent assay; GCT: granulocytotoxicity assay.
[b] Dutch values in parentheses.

and by largely different techniques than the HGA series; the two systems are apparently unrelated (see references in Table I). The other antigens included in Table I have not been directly compared with the former, and may or may not represent different antigens. The techniques used to detect granulocyte antigens, the immunoglobulin class of the anti-granulocyte antibodies, and the gene frequencies of the granulocyte-specific antigens are also shown in Table I. Population studies in different ethnic groups have yielded slightly different gene frequencies (Table I).

Methods used to detect cell surface antigens. The various methods which have been used to detect alloantigens present on the surface of human granulocytes all rely on the same basic principle: granulocytes are isolated from whole human blood and then reacted with antibodies (1–34). The methods used to detect the reaction or binding of antibodies to the antigens are described in this section.

In the leukocyte agglutination test (LAT), the clumping of antibody-sensitized granulocytes is an active property of the cells themselves which is inhibited by heating (1) and cytochalasin B (4). After the granulocytes are coated with antibody and treated with ethylenediamine tetraacetate (EDTA), which prevents nonspecific clumping, the living cells form pseudopods and move toward each other until they contact. The NA-NE and 9a antigens were all detected with this technique (1, 3–10, 18, 21).

The granulocyte cytotoxicity test (GCT) is based on the observation that a normal living granulocyte will exclude a dye such as trypan blue, while a cell sensitized by antibodies in the presence of complement, and thereby damaged, will allow the dye to enter. Modi-

fications of this technique have included the use of a microdroplet method using much smaller amounts of test serum (microgranulocytotoxicity) (17–19), and the use of double-fluorochromatic vital staining to distinguish live, unaffected cells from those damaged and killed by antibody and complement (13).

Because cell viability is crucial in the LAT and GCT test, fresh blood and gentle isolation techniques are necessary for accurate results. Some techniques involve pretreatment of granulocytes with papain (14) or incubation of the granulocytes in methyl cellulose (20). Granulocytotoxic antibodies of the IgG class (12) and IgM class (18) have been described.

Indirect immunofluorescence (IF) has also been used to detect antibodies reacting with antigens on the granulocyte surface (8–10, 21). This technique is complicated by the fact that granulocytes have Fc receptors which nonspecifically bind immunoglobulins (Ig). Consequently, when a fluorescein-labeled anti-human immunoglobulin is used to detect the presence of antibodies on the granulocyte surface, immunoglobulins which are either specifically bound to surface antigens or nonspecifically bound to Fc receptors react. Nonspecific adherence of antibodies to the granulocyte cell surface can be prevented by pretreatment of granulocytes with 1% paraformaldehyde (PFA) (21). Therefore, indirect immunofluorescence can be used to detect surface antigens, which are not affected by the treatment with PFA. The blocking effect of PFA is theoretically caused by its increasing the negative charge of the cell membrane. Antibodies of all classes can thus be detected by immunofluorescence using class-specific antiglobulins. Fluorescein-labeled Fab fragments of anti-human immunoglobulin molecules have also been used (10).

Other assays which depend on the functional characteristics of granulocytes have been employed to detect leukocyte antigens; these tests have the advantage of revealing more about the actual functional characteristics of granulocyte antigens [cited in (3, 4)] (24–27). Examples of such tests are antiglobulin consumption, immunophagocytosis, and the staphylococcal slide test [cited in (3, 4)] (24–27). The last test is particularly interesting because of its ease. Instead of using a fluorescein–antiglobulin conjugate to label antibody-sensitized cells, whole staphylococci containing protein A, which binds to IgG, are used. Granulocytes are isolated by allowing them to adhere to a glass slide. Following sensitization with an antiserum, staphylococci are applied (24, 26). Light microscopic examination is then used to determine if the staphylococci are adhering to the granulocytes due to the reaction between the Fc region of granulocyte-specific antibody and the protein A of the staphylococci. Although these functional tests are not used as frequently as the LAT, GCT, and IF, they have detected clinically significant antibody-related immunoneutropenia (25, 26).

In summary, the three methods most commonly used to detect granulocyte surface antigens are the LAT, GCT, and IF. A number of investigators have used more than one of these methods at the same time, thus facilitating comparisons (21, 23, 28–31). Immunofluorescence has been shown to detect all antigens detected by cytotoxicity, but a small percentage of antigens (e.g., 9a) demonstrable by the LAT are not detectable by IF (1). It is hypothesized that these few antigens bind antibody very weakly, so that although agglutination occurs, the antibodies are washed away in the IF assay (21). No one technique can be considered 100% accurate in the detection of all antigens with a particular antiserum; a combination of techniques is needed for absolute results.

Sources of sera used to identify antigens. Probably the most difficult part of defining the surface antigens of granulocytes is obtaining antisera which are specific for these antigens. Because the antigenic specificities of interest are allogeneic, animal sources of antisera cannot be used, and the sera must come from humans.

The investigation of neonatal neutropenia led to the discovery of the first granulocyte-specific antigen, NA1 (1, 3, 4). The antibodies to granulocytes which develop in pregnant women due to fetal/maternal incompatibilities can result in a transient neutropenia in the newborn, similar in many respects to Rh hemolytic disease of the newborn (5). Although the sera of these women are useful reagents for defining granulocyte alloantigens, only 0.1 to 7% of multiparous women develop these antibodies; the frequently concomitant

TABLE II. MURINE MONOCLONAL ANTIBODIES WHICH REACT WITH HUMAN GRANULOCYTES

Monoclonal antibody	Cells which react with the antibody	Ref.
MA OKM 1	Granulocytes, monocytes	(36)
MA Mo1	Granulocytes, monocytes, null cells	(37, 38)
MO1, MY903, MY904, MY3, MY4, MYL, MY8	Human and chimpanzee granulocytes; many also react with granulocytes of other primate species	(38)
FMC 10-13	Neutrophils and eosinophils	(39)
MY1	Granulocytes, myeloid leukemic cell lines	(40–42)
MA 3C4	Granulocytes, ductal epithelial cells	(43)
1G10	Granulocytes, some monocytes	(44, 45)
T5A7, L4F3, L1B2	Granulocytes, monocytes	(44)
PMN7C3	Granulocytes (inhibits zymosan activation of oxidative metabolism)	(46)
31D8	Subpopulation of granulocytes	(47)
B9.8, B13.4, B34.3, B43.4, B48.4, and others	Granulocytes, other leukocytes (see article)	(48)
AHN 1-6	Granulocytes, some leukemia cell lines	(49)
VEP13	Granulocytes, large granular lymphocytes	(50)
1B5, 4D1	Neutrophil subpopulations	(51)
24.1 (blocks J-5)	Cells bearing cALLA: granulocytes, skin fibroblasts, normal cultured marrow, some leukemia cell lines	(52)
80H.1, 80H.3	Granulocytes, monocytes, some myeloid cell lines	(53)
80H.5	Granulocytes, some myeloid cell lines	(53)
OKM1, LeuM1, LeuM2, LeuM3, MO-1, MO-2	Granulocytes, monocytes	(54)
CD11[M, G, u]: MO1, B2.12, M522	Monocytes, granulocytes, some bone marrow cells, some leukocyte malignancies	(55)
CDw12[M, G, u]:20.2, M67	Monocytes, granulocytes, some leukocyte malignancies	(55)
CDw13[M, G, u]: My7, DU-HL60-4, MCS.2	Monocytes, granulocytes, some leukocyte malignancies	(55)
CDw15[G, u]:BOH.3, B13.9, MCS.1, 82H7, FMC12, FMC13, WM37, DU-HL60-1, FMC10, WM27, WM30, G1120, TG8, WM38, TG1, DU-HL60-3, G2, B4.3, VIMD5, WM41, 1G10	Granulocytes, some bone marrow cells, some leukocyte malignancies	(55)

presence of HLA antibodies, which may interfere with tests for granulocyte antigens and antibodies, further limits the number of useful sera from multiparous women (19–21).

Rare patients who develop autoimmune neutropenia have provided strongly reacting, monospecific antisera for detecting granulocyte antigens (8–10). For example, the antigens ND1 and NE1 were detected with such antisera (8–10). Another source of sera is multitransfused patients who have been, in effect, immunized with the granulocytes of other humans. Although these transfusions have been matched for ABH specificities, other antigenic differences exist, including human leukocyte antigens (HLA) and granulocyte antigens. Because these sera may contain antibodies to a variety of HLA and granulocyte antigens, their utility is limited (33).

A possible technique for producing granulocyte-specific antisera would be the planned immunization of volunteers with granulocytes from a donor who is matched with respect to HLA and other blood cell antigen systems. Although the immunization of volunteers has been useful in the production of HLA-A and HLA-B lymphocytotoxic antisera, it has not been as successful in producing HLA-DR lymphocytotoxic antisera (35).

The latest technique used to produce monospecific sera is the preparation of monoclonal antibodies to granulocyte antigens. The application and success of this new technique in defining granulocyte antigens are discussed in the next section (36–55).

Monoclonal antibodies to granulocyte antigens. Many different monoclonal antibodies have been made which react with human granulocyte antigens. Monoclonal antibodies have defined differentiation and possibly subpopulation antigens present on granulocytes, and a partial list of these is provided in Table II (36–55). A comprehensive review of this rapidly expanding area is beyond the scope of this article; the reader is referred to the individual references in Table II for more details.

One of the first monoclonal antibodies produced which reacts against human granulocytes recognizes an antigenic determinant called My-1 (41, 42, 48, 49). The My-1 "myeloid" antigen appears on granulocytes and their precursors in bone marrow, but has not been detected on lymphocytes, monocytes, platelets, or red cells. In the normal adult human, it is specific for granulocytes (41, 42, 48, 49).

The molecular identity of the My-1 antigen has been found to be a sugar sequence in lacto-*N*-fucopentaose III (LNF III), a glycolipid in the cell membrane (41). This sequence, which is also found in several higher glycolipids and in glycoproteins, contains the epitope which is recognized by anti-My-1 monoclonal antibodies (41). The My-1 polysaccharide sequence found on granulocyte membranes is apparently highly immunogenic in mice (41, 42, 48, 49). In contrast, antibodies to this specificity are not commonly seen in humans and human disease; instead, antibodies to NA1 and other antigens are seen (25).

The My-1 antigen appears on granulocytic precursor cells at the myeloblast–promyelocyte stage of maturation, coinciding with primary granulation, but not at the earliest stage of commitment to the granulocyte lineage, the bipotential granulocyte/macrophage colony forming cell (42). My-1 can therefore be considered a "differentiation" antigen which marks the differentiated granulocyte but not the pluripotential marrow stem cells from which it arises. Other monoclonal antibodies which can serve as differentiation markers have also been produced. The antigens with which these antibodies react appear later during the maturation sequence, and are also present on cells of the monocyte lineage. For this reason, they are useful as markers for different stages of maturation, but not as granulocyte-specific markers (48).

Other investigators have succeeded in producing two monoclonal antibodies which are granulocyte-specific and apparently identify different subpopulations of these cells (51). These monoclonal antibodies react with 51 and 57%, respectively, of peripheral blood neutrophils. They are believed to react with an antigen different than My-1. Although these two antibodies react with almost the same subpopulations of neutrophils, they appear to be distinct. Whether or not these antigenic markers identify functionally different populations remains to be tested, but it is known that neutrophils do show differences in Fc receptor function, bactericidal activity, and chemotaxis (51).

A monoclonal antibody which detects an antigen shared by granulocytes and large granular lymphocytes (LGL) has also been produced by immunizing mice not with granulocytes, but with LGL (50). This antigenic marker appears on these two cell types and a small percentage of T lymphocytes, but not other cells. The LGL are of interest because these cells are natural killer cells, which serve, not unlike neutrophils, as a surveillance mechanism in the body's defense systems against viruses and tumors.

Therefore, monoclonal antibodies which have defined granulocyte antigens include those which are specific for granulocytes, such as My-1, and those which detect antigens which are shared with other cells of the hematopoietic system, such as the LGL-shared antigen (36–55). The My-1 antigen, although normally found only on granulocytes, is not confined solely to them; it has also been shown to be present on human lung cancers and other tumors (41, 48). The stage-specific murine embryonic antigen (SSEA-1) has also been shown to be identical to the antigen defined by My-1 (41). Whether the My-1 (SSEA-1) antigen accounts for any of the properties of granulocytes has not been determined, but granulocytes treated with My-1 antibody exhibit decreased chemotaxis and degranulation. One may speculate that the My-1 antigen may have something to do with a shared property of granulocytes and tumor cells: the ability to move within tissues.

Migrating embryonic cells, metastatic cancer cells, and infection-fighting granulocytes certainly have this ability in common.

The detection of granulocyte antigens reactive with monoclonal antibodies has been primarily through the use of immunofluorescent techniques and the fluorescence-activated cell sorter (FACS). These techniques necessitate the use of costly reagents and equipment which make widespread use difficult. Alternatively, the enzyme-linked immunosorbent assay (ELISA) does not require as many cells or special equipment. A sensitive ELISA technique has recently been described which detects monoclonal antibodies directed against human granulocytes and myeloid differentiation antigens (53).

Monoclonal antibodies and leukemia. The use of monoclonal antibodies to study human granulocyte antigens may soon extend directly into clinical usage, as a means of phenotyping and even treating leukemias (Table II) (52, 54–57). In the same way that monoclonal antibodies are produced against normal cells, they can also be made against antigens found on the surfaces of leukemic cells. In the event that a specific antigen is found on leukemic cells which is absent on normal cells, a monoclonal antibody could conceivably be produced and used to help the immune system locate and destroy malignant cells only.

Although myeloid monoclonal antibodies have not been widely used for therapy, monoclonal antibodies to T lymphocytes (e.g., OKT3) have been used for prophylactic T-cell depletion of bone marrow prior to grafting, in order to prevent graft vs host disease (58). Other monoclonal antibodies (e.g., J5) have been used to treat various leukemias and lymphomas (56).

One of the earliest monoclonal antibodies made to a leukemia cell line detects the common acute lymphoblastic leukemia antigen (cALLA). This antigen was at first thought to be specific for malignant cells of a particular type of leukemia, but was later discovered on a variety of other human tissues, including normal mature granulocytes (52).

Many other monoclonal antibodies have been produced by immunizing mice with a wide variety of malignant cell types, and most have yielded antibodies which are found not only on malignant cells, but on normal cells as well. An example is the antibody to My-1 produced by immunization with lung or colorectal carcinomas, or with normal granulocytes (41, 42). Thus these malignant cell lines express the antigens found on normal but different cell types. The My-1 antigen may be of particular interest because although it has been found on a number of malignant cell types, including leukemias, it is not found on stem cells in bone marrow (42). Consequently, although administration of the monoclonal My-1 antibody would be expected to affect granulocytes as well as malignant cells, the unharmed marrow stem cells might replenish the normal granulocytes.

An experiment of this nature was performed in mice, but a multispecific antiserum to the K-562 cell line was used instead of a monoclonal antibody (57). Human leukemia cells were transplanted into immunodeficient mice, and following treatment with the antibody, malignant growth was suppressed by both direct antibody cytolysis and antibody-dependent macrophage cytotoxicity (57).

Monoclonal antibodies and evolutionary relationships. Another application of monoclonal antibodies specific for human granulocyte antigens is in the study of antigenic changes occurring through evolution. A panel of myeloid-specific monoclonal antibodies has been used to compare primate cell antigens to those of humans (38). As expected, closely related species with recent evolutionary divergence show a high degree of similarity, while those more distantly related have more antigenic differences. However, even somewhat distantly related species still share some antigenic determinants, indicating homology. For example, the chimpanzee shares more human myeloid membrane human antigenic markers than does the slow loris, an early primate (38). The biological reason for the highly conserved nature of granulocyte-specific antigens is unknown.

Clinical significance of granulocyte antigens. These antigens were initially of clinical interest because their reactions with specific antibodies were suspected of causing many febrile blood transfusion reactions not due to ABO or Rh incompatibility (1, 25). These antigens have also been implicated in au-

toimmune neutropenia and in neutropenia of the newborn due to maternal antibodies (1, 3–10, 25).

Granulocyte transfusions. Leukocytes harvested from donors with chronic myelogenous leukemia or normal people premedicated with corticosteroids to increase the yield of granulocytes have made transfusions to granulocytopenic patients possible (59). The efficacy of granulocyte transfusion therapy in management of infections depends on a sufficient dose of granulocytes being given to the recipient and, in some cases, on the presence or absence of preformed antibodies to granulocytes in the recipient (25, 59–65). In one study, McCullough *et al.* found that granulocyte agglutinating antibodies, but not granulocytotoxic or lymphocytotoxic antibodies, altered the fate *in vivo* of indium-111-labeled granulocytes (64).

Recipients of these transfusions include primarily leukemia and other cancer patients whose marrow is affected by cytotoxic drugs and thus are variably immunosuppressed. In these cases, the transfusion of granulocytes which fight infection may be helpful, particularly when the infection is not responding to antibiotics (25, 59). However, these transfusions must be administered as often as daily because of the short lifespan of granulocytes; consequently, a concern over possible immunologic reactions has emerged. In addition, severe pulmonary reactions may result from granulocyte transfusions, especially when given in conjunction with amphotericin B (65, 66). Taken together, all these factors have led to more restraint in the use of granulocyte transfusions (25, 59).

Bone marrow and other transplants. The major concern in transplantation, as in transfusion, is the avoidance of an undesirable immune reaction to foreign antigens. For this reason, the HLA system (MHC) of major human histocompatibility antigens has been used to crossmatch donors and recipients of organs and bone marrow. Although the HLA system is the major histocompatibility barrier in bone marrow transplantation, the fact that graft versus host disease can occur in the HLA-identical setting indicates that there are other non-MHC antigens which are also of significance (67–72). Granulocyte antigens may be of interest in bone marrow transplants because these antigens are normally found on cells of marrow origin; however, the stem cells often lack mature differentiation antigens. The incidence of granulocyte antibodies in other types of transplants has been studied as well (67–72).

The leukocyte group 5 antigenic incompatibilities have no significant effects on the outcome of marrow transplantation (67) and only a slight, if any, effect on the outcome of skin grafts (67, 71). The granulocyte-specific antigen NA1 has also been shown, in at least one case study, to have little or no effect on marrow transplantation. In this case, NA1-positive marrow was transplanted into a patient who already had NA1 antibody present, but who was HLA-identical to the donor. The transplant was successful, apparently because the marrow stem cells which were transplanted did not exhibit the antigen (72). In another study involving kidney transplants, there was no correlation between the existence of preformed granulocytotoxic antibodies to the donor's granulocytes and the success or rejection of a grafted kidney. This result might be expected since the kidney may not exhibit the granulocyte antigens (70).

In summary, the role of non-HLA antigens present on granulocytes and other cells is unknown with respect to tissue transplantation (25, 69). Although there is one study in which antibodies to antigenic determinants on monocytes, as identified by an antibody-dependent cell-mediated cytotoxicity test, were correlated with bone marrow graft rejection (69), other studies have found no correlation between non-HLA myeloid antigenic incompatibilities and the rejection of bone marrow or other grafts (67, 68, 70–72). The fact that granulocyte antigens are limited to the granulocyte series, and only mature cells of that lineage, makes it unlikely these antigens play a major role in tissue transplantation.

Autoimmune neutropenias. Unlike transfusion and transplantation reactions, the cause of autoimmune neutropenia has been definitely linked to granulocyte-specific antigens (73–86). In fact, many of the known granulocyte antigens have been defined by the sera of autoimmune patients (5, 75, 77).

Idiopathic autoimmune neutropenia or

granulocytopenia is analogous to other autoimmune diseases, such as autoimmune hemolytic anemia and thrombocytopenia. These disorders can occur alone or in conjunction with other diseases such as systemic lupus erythematosus (SLE) or Felty's syndrome. In one study of the latter disease, 13 out of 15 patients had antibodies against their own leukocytes (74). SLE and asthma patients have also been shown to have a significantly higher incidence of granulocytotoxins (53%) than the normal population (about 10%) (73, 79). This research also suggested that some granulocyte antibodies which were thought to be caused by immunization (i.e., transfusion) were actually autoimmune in nature.

The granulocyte-specific antigens which have been defined with sera from autoimmune patients include NA1, NA2, and NB1 (5, 75, 77). NA1 and NA2 have both been described in young children with neutropenias (75, 77). In both cases, all evidence pointed to the existence of autoantibodies, as opposed to antibodies resulting from maternal diffusion or immunization. The respective antigens in both cases were detected on the patients' granulocytes after the autoimmune disease subsided, either spontaneously or due to steroid therapy (75, 77).

These granulocyte autoantibodies have also been demonstrated by agglutination, cytotoxicity, and immunofluorescence assays in adults with idiopathic neutropenia (4). The sensitive immunofluorescent technique is theoretically preferable because it can detect the presence of antibody on cells taken from the patient, analogous to the direct Coombs test used to diagnose autoimmune hemolytic anemia (4).

Family studies and HLA typing have also confirmed granulocyte-specific antigens, and not other antigen systems, as the target of autoimmune antibodies (78). Antigens which are shared with other hematopoietic cells such as monocytes have also been implicated in neutropenia (80). In some cases, the antibody may be specific for a non-HLA antigen shared by granulocytes, monocytes, lymphocytes and marrow stem cells (81). The antibodies detected in these cases all have analogues among the monoclonal antibodies discussed earlier, which include granulocyte-specific, granulocyte- and monocyte-specific, and granulocyte-, monocyte-, and stem-cell-specific antibodies. Whether or not these cases are detecting the same sets of antigens has not been shown.

Neonatal neutropenias. Neonatal neutropenia, due to fetal–maternal incompatibility, was the clinical disorder which first led to the discovery of granulocyte-specific alloantigens (82–84). In the case studies, three of four infants in one family had a neutropenia which lasted several weeks after birth, and after serological analysis and use of the agglutination test, the existence of the NA1 antigen, present on the children's but not the mother's neutrophils, was discovered (82). Since that time, other antigens have been defined by the sera of multiparous women who have been immunized by fetal granulocytes (82–84), as shown in Table 1.

The pathological condition of neonatal neutropenia is entirely analogous to hemolytic disease of the newborn. In both cases, the antibodies produced by the mother to paternal/fetal antigens are able to cross the placenta and damage cells in the fetus. Erythroblastosis fetalis results in anemia, while neonatal neutropenia results in agranulocytosis and consequent loss of resistance to infection, which can be life threatening. The placenta is theoretically unable to screen out and absorb these antibodies because they are granulocyte specific and are not present on placental tissues (4).

Leukemias. Normal granulocyte alloantigens have also been located on chronic myelogenous and acute myeloblastic leukemia cells, as well as on the K562 cell line, which is also of chronic myeloblastic origin (85). Acute lymphoblastic leukemias were shown to react only very rarely against the granulocyte-specific sera, and chronic lymphocytic leukemia cells did not react at all (85). This situation is paralleled once again by the studies with monoclonal antibodies to granulocyte antigens, which have a similar incidence in myeloid leukemias.

Another study compared the frequency of leukocyte group 5 antigens in normal and leukemic people (86). Among controls, the 5a gene frequency was 0.09, while in a group of acute lymphoblastic leukemia patients, the frequency was 0.38. Thus, there was a significant association between this antigen and

the occurrence of this leukemia. The appearance of the antigen was not caused by the disease, and other types of leukemia did not show any correlation with the presence of this antigen (86). This situation is not unlike that of the HLA system, in which the presence of certain antigens is correlated with an increased incidence of specific diseases (87).

Summary. The biological reason for the polymorphism of granulocyte-specific antigens is unknown. Clinical complications due to granulocyte antigen–antibody reactions cannot yet be predicted with certainty. Thus reactions following granulocyte transfusions may occur, despite cross-matching (25, 59–66). Autoimmune disorders and maternal/fetal incompatibility have been traced to known granulocyte antigens, and sera from patients with these disorders have been used to define many of these antigens (73–86).

One of the main difficulties in deciphering the system of granulocyte antigens has been the limitations of the various testing procedures used. None of the techniques yet discovered, including indirect immunofluorescence, has been able to detect all the antigens which other tests detect (28–31). This fact suggests that these tests may be inadequate and could explain their lack of predictive value. A panel of known cell types is also unavailable, because the viability of granulocytes following current cryogenic procedures is unpredictable and often disappointing.

The lack of high-titered monospecific antisera directed against antigens has also been a major block to research. Efforts to produce monoclonal antibodies, bypassing this block, have detected species-specific, subpopulation, differentiation, and leukemic antigens, but not the clinically interesting alloantigens (36–57). The development of more sensitive tests and monoclonal antibodies specific for granulocyte alloantigens could lead to a better understanding of granulocyte antigens and be helpful in treating disorders involving these antigens.

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