Current Concepts of Leukemia and Lymphoma: Etiology, Pathogenesis, and Therapy

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...e cellular change (phenotypic) leading to leukeniia niay ...olve a disorder of leukocyte maturation, but the etiologic ...ecular change (genotypic) remains unknown. We present ...dence here that human leukemic cells contain type-C ...al information and consider the possible significance of ... observation in the context of a working hypothesis. We ...examine reticuloendothelial neoplasms in the light of ...ewer immunologic, cytochemical, and ultrastructural ...ethods for identifying cells of the T-lymphocytic, B-...mphocytic, and monocyte-macrophage systems. Use of ...ese methods has led to a challenging concept of ...alignant lymphomas as neoplasms of various anatomic ...d functional compartments of the immune system. ...unctional studies, although still in their inception, have ...ready provided provocative clues in the etiology and ...athophysiology of these disorders. Advances in laboratory ...esearch have been paralled by dramatic changes in clinical ...ncology, as evidenced by trends in the treatment of ...cute lymphocytic leukemia, acute myelogenous leukemia, ...odgkin's disease, and diffuse histiocytic lymphoma.

Dr. COSTAN W. BERARD*: We shall present a brief over-... of recent advances in the study of leukemia and ...mphoma. Our first speaker will discuss ideas on the ...thogenesis and etiology of human leukemia.

The Origin of the Lymphomas

Dr. Rohert C. Gallo†: There is very little information ... the causation of human lymphomas, except for Bur-...itt's lymphoma. We now have considerable evidence that Burkitt's lymphoma is caused by an infectious DNA virus, ...he Epstein-Barr virus. Final proof may depend upon a specific therapeutic result. Since this subject has been reviewed recently by experts (1), I will not discuss it further. Instead, I will focus on our ideas on the origin of leukemias, especially acute myelogenous leukemia.

Different questions can be asked about the origin of leukemias. Some relate to pathogenesis, that is, the physiologic abnormality and cellular change that leads to it—in short, the phenotypic change. Of concern to molecular biologists is the nature of the information responsible for the cellular change, the genotypic change (if any) responsible for the disease.

PATHOGENESIS

Physiologic Change: Most workers believe that acute myelogenous leukemia results from a physiologic abnormality in the process of differentiation of the granulocyte precursors (myeloblasts) to the mature polymorphonuclear leukocytes or between stem cells and committed granulocyte precursor cells. This is often viewed as a "block" in differentiation, and it has been discussed in detail (2-6). Studies from many laboratories have shown that substances are released into the medium from some cells and that, when added to hematopoietic cells, they can induce modest temporary growth and differentiation of these cells, especially on a solid matrix. Such substances have been called colony stimulating activity and macrophage granulocyte inducer, and the assay systems for them were first developed by Sachs (7) and by Metcalf, Bradley, and Robinson (8). They seem to be heterogeneous, and those that have effects on human cells have not been chemically characterized. Some studies with these materials have indicated that the phenotypic abnormality in acute myelogenous leukemia is reversible, that is, the cells respond to the putative regulator molecules and differentiate (7). Others believe that the arrest in differentiation, at least for most cells, is irreversible. The emerging hypothesis is that the population of leukemic cells is heterogeneous in the ability of the cells to respond (7, 9), thus raising an important question: Is the genetic change (if any) that leads to leukemic transformation present in

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only some of the cells we call leukemic, or is it present in all cells. but expressed (DNA→RNA) to a varying degree in different leukemic cells'! In either case, these and other studies have shown that at least some of the acute myelogenous leukemia cells do respond to these stimuli. In addition. Gallagher and colleagues (10) recently discovered a new "factor" that seems to be completely specific for niyclogcnous leukemia cells. This factor was obtained from conditioned media from one particular human embryonic cell line. It promotes continuous exponential growth of thrsc cells and maturation of at least sonic of them in liquid suspension culture. It has no cllcct on acute or chronic lymphocytic leukemia cells. normal bone marrow, or normal peripheral blood cells. This factor differs from the colony stimulating activity in that [1] it is specific for leukemic cells, whereas colony stimulating activity works best or more predictably on normal cells; [2] it induces exponential growth: [3] its effect can be maintained continuously; and [4] it works on cells in suspension and does not promote colony formation in the agar plate system. The difference between the responses of the leukeniic and normal cells again emphasizes a cellular difference between the two. Since sonic of these results were obtained with leukemic cells containing marker chromosomes and since normal cells do not respond to this factor, we belicve these results substantially add to the evidence that at least sonic acute myelogenous leukemia cells can hc induced to differentiate.

Cellular Defect (Phenotypic Change). What is the precise cellular change responsible for these functional differences between normal and leukemic cells? Despite many morphologic, immunologic, and biochemical studies, this has nnt been pinpointed in acute myelogenous leukemia or in any other neoplasia. For theoretical reasons many workers suspect the membrane. The differences in the response of nornial and myelogenous leukemic cells to the growth- and differentiation-inducing factors could also be viewed as differences in membrane recognition of these factors. Ilowevcr, by no means are other mechanisms excluded. Sachs (7) reported that murine leukemic and other transformed cells respond differently to certain lectins than do normal cells, suggesting that the surface of transformed cells is altered. However, I ani not aware of any substantial study of this with human leukcniic cells. I think the best evidence that the surface of leukemic cells differs from normal resides in the recent results of Billings and Terasaki (11) and particularly of Brown, Hogg, and Greaves (12) on isolation of a leukemic specific surface antigen.

FAC TORS INFLUENCING THE INCIDENCE OF LEUKEMIA

Several factors arc associated with an increase in the incidence of leukemia, and, since they are well known and often reviewed, I will mention them only briefly: radiation, some chemicals (especially benzene), probably some antitumor agents. chloramphenicol, and certain congenital abnormalities often associated with chromosome abnormalities such as Down's syndrome and Bloom's syndrome, ataxia telangiectasia, and Fanconi's anemia. Heredity can also he very important; there arc several reports of families with an extraordinary incidence of leukemia. The most striking evidence of the importance of heredity is the very high incidence of leukemia in an identical twin of a patient with leukemia. There are several theoretical possibilities by which hereditary abnormalities could lead to an incresse in susceptibility to the disease—for example, after the primary change occurs, an inherited immune deficiency could lead to an increased chance of survival of transformed cells. Similarly, it is also possible that chemicals, radiation, and congenital changes work through secondary mechanisms, that is, by diminishing host resistance. However, these and hereditary factors could theoretically also be primary causes (directly leading to cell transformation). There is no evidence to say one way or the other.

IDEAS ON THE NATURE OF THE INFORMATION INVOLVED IN LEUKEMOGENESIS

Class 1 and Class 2 Viruses. Viewed broadly, there are many causes of leukemia However, they may all involve one final common molecular mechanism. Does it involve only derepression of normal unaltered genes leading to cells akin to the fetal state, or does it involve gene modification through change (mutation or deletion) or by acquisition of new information, as by infection?

Most leukemogenic agents can cause genetic change, but we do not know if this is essential for development of the disease. I describe below our ideas and some data related to these questions.

Two major groups of viruses cause neoplasia in animals the DNA tumor viruses and the RNA tumor viruses Regarding the leukemias, the accumulated data strongly incriminate the type-C RNA tumor viruses. These viruses seem to have originated from genes of normal cells (13 15). Some have remained under host control and are often called endogenous. For several reasons. we prefer the term class I type-C virus (15). We think information from these viruses is involved in normal development (16, 17), and this might include the normal differentiation process of hematopoietic cells (17). When one hybridizes the RNA genonie of these viruses to DNA of the normal uninfected host animal, an excellent match is obtained Although derived from a given host cell, they arc usually not infectious for that cell, a characteristic that Levy (18) has called xenotropic. There is no clear evidence that these viruses are oncogenic, at least not in their host, and we belicve that, unless genetically altered. they are not (Figure 1). Some examples are RAV, of chickens, the endogenous murine viruses. RDI 14 of cats, the guinea pig endogenous virus, and the only endogenous virus so far isolated from primates, the endogenous baboon type-C virus. It could be argued that the AKR virus of mice is endogenous. and it is leukemogenic. However, this animal is inbred for high-incidence leukemia. Probably the endogenous (class 1) "virogenes" of this animal have been modified. We have proposed that if the class I endogenous "virogenes" are modified by mutation (heredity. chemicals, radiation, or by an infecting virus that can integrate DNA into this region). this may lead to improper differentiation and growth control (14).

We define a class 2 type-C virus as one that contains in its RNA pcnomc at least some nucleotide sequences detectably different from its host (IS). If a class 1 virus escapes host control and hccomes infectious. it may undergo genetic change (15) and heconie a class 2 virus for its original host. Or, if crivironmental events result in direct genetic change of the class 1 endogenous "virogene," and if this should hc fully expressed (DNA→RNA), then it too would become a class 2 virus (Figure 1). In this sense, the environmental event would have caused the disease and also led to virus production. However, once formed, a class 2 virus could transmit the leukemogenic information to other cells, and in some instances it might become infectious and therchy he the natural primary cause of the disease. A class 2 virus is, in fact, usually oncogenic and infectious. Class 2 viruses can indeed he the natural infectious cause of leukemia, as is often the case in cats and occasionally seems to he the case in birds and at least in some primates. The infection can be congenital (avian leukemia) or from contact (feline leukemia). The leukemia and sarcoma viruses of chickens, mice, cats, and primates are examples of the class 2 viruses. Table 1 summarizes some of the properties of these viruses.

Primate Type-C Viruses: Primate type-C viruses, class 1 and class 2, were recently isolated. The baboon endogenous virus seems to be a typical class 1 virus (19). Man may also harbor an endogenous class 1 virus (19), but, if so, it has not yet been isolated. More importantly, a "family" of closely related class 2 type-C viruses has been isolated from several gibbon apes and one woolly monkey (20, 21). Some of the gibbon isolates have come from gibbons with leukemia (20), and some can induce myelogenous leukemia (22). The woolly isolate (also called simian sarcoma virus) came from a fibrosarcoma. The gibbon

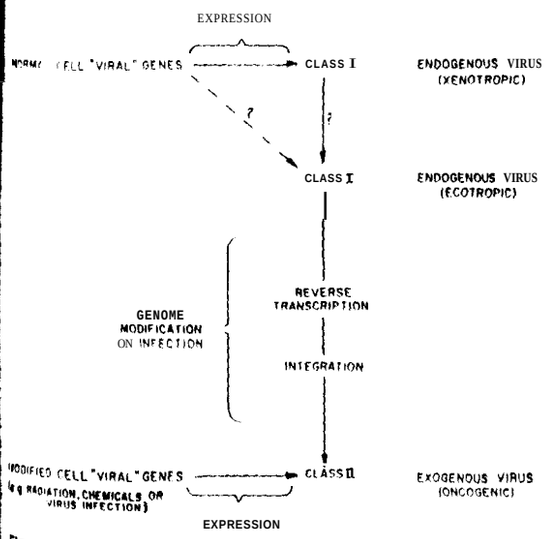

Figure 1. Schematic illustration of the origin of type-C viruses and how they may undergo genetic modification. Shows class 1 viruses as normal gena products and class 2 viruses as modifications of the class 1 viruses. one consequence of which may be the acquisition of oncogenic capability. See text lor discussion.

Table 1. Distinguishing Features Between Class 1 and Class 2 Type-C RNA Tumor Viruses

	Class 1	Class 2
Source	Usually normal tissues; can he neoplastic tissues	Usually neoplastic tissues
Oncogenicity	Generally not known to be oncogenic	Generally known to be oncogenic
Transmission	Usually vertical (germ line) as "provirus"; usually not infectious	Usually horizontal but can infect germ line and can be vertically transmitted
Molecular hybridization	RNA genome complexes to DNA from uninfected cell	RNA genome complexes poorly lo DNA from uninfected cell

virus can apparently he transmitted to other gibbons naturally (without inoculation) and can spread the disease*. Of great interest, most isolates have come from animals inoculated with human material. In a study in Thailand of 195 gibbons (23), 92 animals were injected directly with blood from human patients with malaria (or pawed from gibbon to gibbon). The remaining 103 animals were a control group. Most strikingly, 10 of the injected animals developed leukemias or lymphosarcomas. The first isolates of the gihhon virus came from these animals. None of the 103 members of the control group developed neoplasia. The human blood may have in some way activated type-C virus already present in the gibbons. Alternatively, man may he the source of the virus. The woolly and gibbon isolates ate not endogenous to primates, and they may have had their origin in the distant past in mice, entering primates as an infectious and oncogenic virus (class 2 for primates) (24, 25).

Evidence for Type-C Virus Components in Human Leukemia *(Table* 2): Several reports have described the presence of molecules in human leukemic cells related to analogous molecules of certain animal type-C viruses. They followed from the detection (26) and subsequent partial purification (27-30) of a reverse transcriptase and of viral-related nucleotide sequences (see below). Later reports (31, 32) dcscrihed detection of reverse transcriptase in virtually all patients with leukemia, including those in remission. This was done by the simultaneous detection assay (33), which measures a reverse transcriptnse-like activity and a high molecular weight RNA (hence viral-like). Our results indicate a more restricted dicfribution. since we find the enzyme in only approximately 30% of patients with leukemia. However, for a positive score we do use this endogenous activity, but we also require partial purification and characterization of the enzyme. Use of the activity assay alone is, of course, more sensitive, but certainly the requirement for enzyme isolation and characterization gives added assurance that one is measuring reverse transcriptase. On the other hand, requirement of enzyme isolation could lead to false-negatives.

A few years ago, Todaro and Gallo (34) reported that in some cases of acute myelogenous leukemia the reverse

* KAWAKAMI T: Personal communication.

Table 2. Summary of Evidence for Type-C Virus Related Information in Some tiunian Leukemic Cells

Viral Related Component	References
Reverse transcriptase (biochemical)	26-32, 34, 44, 47
Reverse transcriptase (immunologic)	29, 30, 34, 45
P30 protein	36
Viral-related nucleotide sequence	31, 32, 36-40, 126,*
Miscellaneous viral-related proteins	†
Release of virus or viruslike particles	39, 41, 42, 45-47

* TAVITIAN A: Personal communication.
† BOLOGNESI D: Personal communication

transcriptase is antigenically closely related to the reverse transcriptases **of** the gihhon ape leukemia **and** the woolly monkey sarcoma viruses. Others **(29,** 30) later observed this in several more patients. The reverse transcriptase that we *have identified* in adult acute myelogenous leukemia cells has conristently been antigenically related **to** the primate *oncogenic viruses. This* **has** not hccn the case in other leukemias. Even in acute myelogenous leukemia our success has hecn limited to *about* 30% **of** cases. In **1975,** Shrrr and Todaro **(35)** detected a protein antigenically related to one of the major structural proteins (p30 protein) **of** the same primate viruses, and Bolognesi* detected viral-related proteins on the surface of leukemic cells. Others (31, 32, 36) found nucleic acids in the cytoplasm of leukemic cells that contained nucleotide sequences related to mouse leukemia viruc RNA. We confirmed these results and extended them to show more homology to the RNA of the woolly monkey virus **(37,** 38), a recent finding **of** Mak and associates **(39) and** of Tavitian†: These results all say that information (complete or partial) related to genomes of animal type-C virus is present in sonic and perhaps all human leukemias. **Al**though there findings do not bear **on** the origin of this infnrmation (cndopenour or acquired hy infection), the detection in *some* cares **of** molecules related to class 2 primate onzogenic viruses suggests that in these the information may have heen acquired. Baxt and Spiegelman (32) and Baxt **(40)** observed extra viral-related sequences in cells from all patients with leukemia that are not seen **in** normal cells. Further, these sequences are related to mouse leukemia virus. **So** far these very important results have not been confirmed.

Isolation of Type-C Virus: Even though type-C virus infnrmation and components may be detected in some leukemias, formation of whole virus must be rare, perhaps because the virus may **he** defective or suppressed. Recently, **we** identified in a single patient type-C virus in **two** separate blood samples and **one hone** marrow specimen obtained at three different periods in the course of the disease (10, **41**). The virus is infectious for many cells (42) and consirtz of two components—one closely related **to** the woolly monkey virus and the second closely related **to** the endogenous type-C virus of baboons (41-43). We call the virus HL23V. In the fresh, uncultured **blood cells** of this same patient, we found reverse transcriptase related to the woolly monkey virus **(44).** In addition, Mak and colleagues (39), studying leukemic: cells in **short** term culture, reported transient release **of** viral-like **particles** that contain RNA with sequences predominantly related to the woolly monkey virus. Although the particles the described have **not as** yet heen shown to bud **from** cell membranes nor to be biologically active, they may represent release of immature viruslike particles previously identified within the cytoplasm of these **cells** (27-32, 36-38, 40, **44).** Rcccntly, Nooter and co-workers **(45)** isolated from a child with leukemic lymphosarcoma a virus that apparently **is** also related to the woolly monkey virus Gabelman and associates (46) isolated a similar virus from **a** patient with chronic lymphocytic leukeniia, and Panem **and** colleagues **(47)** now report that they have isolated a virus closely related to the woolly monkey **virus**-gibbon ape virus "family" from several normal human embryos If verified, this will he a landmark discovery. As mentioned above, in the woman with acute myelogenous leukemia from **whom** we isolated the HL23 virus, we later found a second virus cotnponent. In other studies, Panem and associates also detected this second component, **which** war closely related to the endogenous virus of baboons. We recently detectcd the DNA provirus of the endogenous virus of baboons in the spleen (received postmortem) of our patient (43). This shows that the virus did in fact come from the patient and not from laboratory contamination.

Since these viruses arc not truly endogenous hut rather class 2, we think the results **of** Panem and co-worker, (47) suggest transmission from congenital infection. This could explain the lack of epidemiologic evidence for *infection,* since after congenital infection a virus may remain dormant unless activated by an environmental stimulus. This idea is further supported by a striking clinical observation by Thomas and colleagues **(48)** in Seattle. In their now well-known reports. they descrihcd apparent leukemic transformation of normal donor bone-marrow cells after inoculation into leukemic recipients.

One major problem to date with the viruses that have been isolated is that we have not yet regularly identified a complete provirus (the integrated DNA derived from the virus after RNA→DNA conversion) in human leukemic cells‡. Even if all these observations are correct. the data from **our** results do not indicate that this **happens** in all leukemias or even in all patients with acute **myelogenous** leukemia. Acquisition of type-C viral information may he One of several means of causing leukemia by one molecular mechanism.

Dr. Costan W. Berard: Malignant lymphomas have traditionally been diagnosed and classified solely by morphologic criteria. Early classification schemes were

* BOLOGNESI D. Personal communication.
† TAVITIAN A. Personal communication

‡ As noted above, DNA provirus related to the baboon endogenous type-C virus was discovered in the DNA from postmortem specimens obtained from the wnman with acute myelogenous leukemia (43) from whose cella we isolated a type-C virus (10, 41). Recently, we found provirus sequences of the hahoon endogenous virus in the DNA from uncultured tissues from several (hut not all) other leukemic patients (WONG-STAAL F, GILLESPIE D, GALLO RC: Proviral sequences of baboon endogenous type-C RNA virus in DNA of leukaemic tissues from seven patients with myelogenous leukaemia. *Nature,* In press). The results indicate that viral nucleic-acid sequencer related or identical to those found in the hnboon endogenous virus have heen acquired by some humans. The simplest interpretation is that of an interspecies transmission of this virus In the past from baboon to man.

leukemic cells in short-term release of viral-like particles predominantly related ... Although the particles they were shown to bud from cell ... biologically active, they may ... viral-like particles previously ... of these cells (27-32, 36-38, ... and co-workers (45) isolated ... lymphosarcoma a virus that ... the woolly monkey virus. ... isolated a similar virus from ... leukemia, and Panem ... port that they have isolated a ... woolly monkey virus-ribbon ... viral normal human embryos ... mark discovery. As mentioned ... acute myelogenous leukemia ... virus, we later found a ... other studies, Panem and ... second component, which was ... virus of baboons. We ... portions of the endogenous ... (received postmortem) of ... that the virus did in fact ... not from laboratory con- ... truly endogenous but rather ... Panem and co-workers (47) ... genital infection. This could ... with evidence for infection, ... virus may remain ... an environmental stimulus, ... by striking clinical ob- ... (48) in Seattle. In ... they described apparent ... normal donor bone-marrow ... recipients.

... with the viruses that have ... not yet regularly identified ... DNA derived from ... conversion) in human leu- ... observations are correct, the ... indicate that this happens in ... patients with acute myelogenous ... viral information may be ... leukemia by one molecular ...

Malignant lymphomas have ... and classified solely by ... classification schemes were ... plagued with problems, largely because of diverse use of generic terms such as "lymphosarcoma" and "reticulum cell sarcoma" and lack of agreement regarding the cytologic features of the neoplastic cells in many tumors. In the past decade more detailed subclassifications of both Hodgkin's disease and the non-Hodgkin's lymphomas have been developed and shown to have considerable value for clinicopathologic studies (49). These new schemes, however, are also based primarily on morphologic criteria. With the advent of new immunologic, cytochemical, and ultrastructural techniques, it has been possible to characterize normal cells of the T-lymphocytic, B-lymphocytic, and monocyte-macrophage systems. Recent application of these same methods to the cells of leukemias and malignant lymphomas is leading to dramatic advances in our understanding of the pathogenesis and pathophysiology of these disorders (50). Doctor Jaffe will summarize present knowledge regarding immunologic markers on the cells of these tumors.

Immunologic Markers

Dr Elaine S. Jaffe*: Recent advances in immunology have led to a reexamination of many of the conventional concepts regarding the classification and nomenclature of diseases of the reticuloendothelial system. In the past, the small lymphocyte was regarded as an endstage cell, incapable of further maturation or division. We now know that with the appropriate stimulus, whether it be antigen, mitogen, or neoplasia per se, this cell can undergo a marked morphologic transformation and rapid cell division. Furthermore, the seemingly homogeneous population of small lymphocytes seen in the peripheral blood and lymphoid tissues in fact consists of at least two cell types, the T and B lymphocytes, with distinct origins, functions, and properties (Table 3). These cells, as well as a third class of immunocompetent cells, the mononuclear phagocytes, can be identified by characteristic surface membrane markers (Table 4). These markers are most commonly identified on cells in suspension isolated from either peripheral blood or lymphoid tissues. However, certain of these markers can be identified on cells in frozen tissue sections, specifically receptors for complement, as shown by the binding of EAC (51, 52), and receptors for cytophilic antibody, as shown by the binding of IgG EA (52). All of these methods can be applied to normal as well as neoplastic tissues for the identification of lymphoreticular cells.

These immunocompetent cells have different localizations in the peripheral lymphoid organs, and, in fact, one can view the anatomic compartments of a lymph node as the functional components of the immune system. B cells are distributed in the far cortex, the follicles and the medullary cords. However, B cells in these different sites differ with respect to both their function and their surface markers. The lymphoid follicle is a site of antigen localization (53) and also represents the proliferative arm of the B-cell system (54, 55). The medullary cords, in which differentiation toward plasma cells occurs, comprise the secretory arm of the 9-cell system. The paracortex is the thymic-dependent portion of the lymph node (56) and the site of T-cell recirculation through postcapillary venules (57). Mononuclear phagocytes are distributed primarily in the cortical and medullary sinuses.

Recent studies of the surface characteristics of neoplastic lymphoreticular cells have helped us to understand how many of the malignant lymphomas relate to the above anatomic and functional components of the immune system (Figure 2). I shall now discuss certain selected examples and show how each of the four major compartments manifests itself as neoplasia. Recent evidence indicates that nodular lymphomas are indeed neoplasms derived from the lymphoid follicles (58, 59). The Rappaport classification (60), currently the most clinically relevant and widely used scheme, subdivider nodular lymphomas on the basis of the cytologic composition of the neoplastic nodules. The small cells are termed "lymphocytes" and the larger cells are called "histiocytes" because of their morphologic similarity to normal histiocytes. Studies at the National Institutes of Health (58) and at Vanderbilt University (59) have shown that all of these cells are in fact follicular B lymphocytes with identical patterns of immunologic markers, and that these different cell types probably represent different stages of transformation. The neoplastic cells are characterized by the presence of monoclonal surface immunoglobulin (59) and abundant complement receptors easily demonstrable on cells in suspension as well as in frozen tissue sections (58). Normal follicular B lymphocytes can also be distinguished from other B lymphocytes within a lymph node by the above surface characteristics.

If nodular lymphoma represents the neoplastic equivalent of the lymphoid follicle, then well-differentiated lymphocytic lymphoma or chronic lymphocytic leukemia represents the neoplastic counterpart of the medullary cord

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Table 3. Characteristics of Human B and T Lymphocytes

	B Lymphocyte	T Lymphocyte
Origin	Bone marrow	Bone marrow
Differentiation	Bursa equivalent; (?) liver	Thymus
Function	Humoral immunity	Cell-mediated immunity

Table 4. Identification of Cells by Surface Markers*

	SIg	C3	Fc	E	Ag
T Lymphocyte	∓	∓	‡†	+	∓
B Lymphocyte	∓	∓	∓	−	+
Monocyte-histiocyte	±‡	±§	$-	−	−

* SIg = membrane-bound surface immunoglobulin; C3 = receptor for third component of complement-commonly identified by binding of EAC (sheep erythrocytes coated with IgM and complement); Fc = receptor for Fc portion of IgG identified by binding of IgGEA (sheep erythrocytes coated with IgG antibody) or by binding of aggregated fluoresceinated IgG; E = spontaneous rosette formation with sheep erythrocytes; Ag = presence of specific isoantigens identifiable by preparation of specific antisera.

† Receptor present only on a small population of activated T cells.

‡ SIg is not a cell product and is nonspecifically bound.

§ Receptor present on peripheral blood monocytes but not identified on all tissue histiocytes.

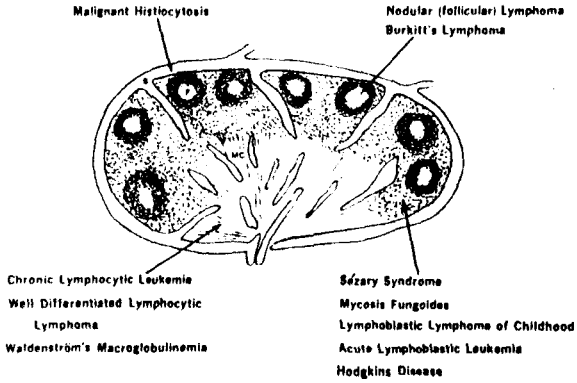


Figure 2. Diagrammatic representation of a normal lymph node, showing the anatomic and functional compartments of the immune system. The malignant lymphomas are related conceptually and functionally to each of the above compartments. S = sinuses; F = follicles: PC = paracortex: MC = medullary cords.

B lymphocyte. These tumors never manifest a nodular growth pattern but always infiltrate in a diffuse fashion. In well-differentiated lymphocytic lymphoma there is a proliferation of homogeneous, small, mature-appearing lymphocytes. These cells do not proliferate rapidly but are highly motile, like a mature lymphocyte, and readily gain access to the peripheral blood. Thus it is readily understandable that most patients with well-differentiated lymphocytic lymphoma are at great risk to develop chronic lymphocytic leukemia, and in fact these two diseases simply represent different clinical expressions of the same neoplastic process.

As expected, markers identified on the cells from well-differentiated lymphocytic lymphoma and chronic lymphocytic leukemia are identical. Studies in our laboratory of 10 cases of well-differentiated lymphocytic lymphoma and 13 cases of chronic lymphocytic leukemia have shown identical patterns of immunologic markers (61). These cells are characterized by the presence of monoclonal surface immunoglobulin, most commonly of the IgM type; however, the staining is usually quite faint. This staining pattern contrasts with that of nodular lymphoma cells, in which bright fluorescent spots appear on the cell membrane, often with spontaneous capping. The cells of well-differentiated lymphocytic malignancies usually have complement receptors, another B-cell marker, but, in contrast to the cells of nodular lymphoma, their complement receptors are characteristically qualitatively or quantitatively deficient, such that they cannot be identified by the frozen section technique. This feature is also seen in the medullary cords, an area populated by plasma cells and lymphocytes presumably differentiating toward plasma cells.

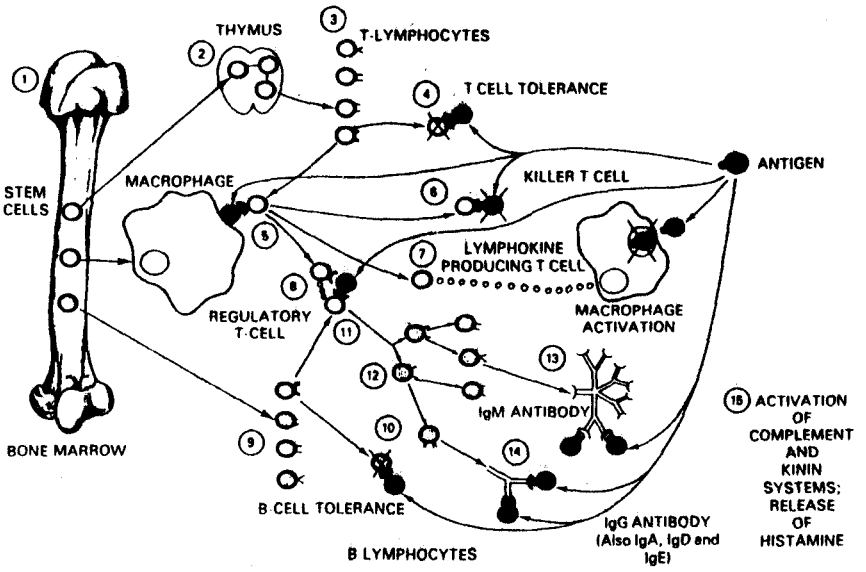
We have mentioned that the medullary cords comprise the secretory arm of the B-cell system. However, in most cases of chronic lymphocytic leukemia and well-differentiated lymphocytic lymphoma. secretion does not occur, and, in fact. hypogammaglobulinemia is observed (62). If in a lymphoma of this rystcm the immunoglobulin moiety is synthesized and secreted into the serum instead of being expressed only on the cell membrane, the patient will have a monoclonal gammopathy, most commonly of the IgM type, and the clinical picture of Waldenström's macroglobulinemia. in this event, the cells usually appear more plasmacytoid.

We turn now to tumors that appear to be of T-cell type, such as childhood lymphosarcoma or diffuse lymphoblastic lymphoma occurring in children and young adults The presence of a mediastinal mass in many of these patients had suggested that these tumors might be of thymic origin (63), and in fact T-cell markers have been seen in a number of cases in vitro (64-66). As one would expect, these tumors also tend to involve the thymic-dependent portions of the lymphoreticular system (67).

The large cell malignant lymphomas (reticulum cell sarcomas) have been termed "histiocytic" in the Rappaport classification because of their morphologic resemblance to normal histiocytes. However, immunologic studies of most cases of histiocytic lymphoma that occur de novo show a heterogeneous pattern, sometimes demonstrating B-cell markers (68-70), sometimes no markers (71), and, rarely, T-cell markers (70, 72). Presumably, these tumors are composed of highly transformed or dedifferentiated cells of diverse origins, and, because of this dedifferentiation, they fail to follow the homing patterns characteristic of better differentiated lymphomas. Markers can consistently be shown in one group; namely, those cases in which the large cell tumor occurs as a progression of a pervious neoplasm in which markers were present. these cases, when they occur as a progression of chronic lymphocytic leukemia, are known as Richter's syndrome, and the cells retain the same surface immunoglobulin as the chronic lymphocytic leukemia cells (70). The same phenomenon can also happen as a progression of nodular lymphoma, and, here again, B-cell markers are retained (67).

To turn to a neoplasm of true histiocytic origin, only one such example is well proven and that is malignant histiocytosis, or histiocytic medullary reticulosis. This disease seems to represent a neoplastic transformation of the sinusoidal histiocytes of the lymphoreticular system. These cells retain the functional capacity of histiocytes and have a marked phagocytic potential commonly exhibited as erythrophagocytosis. The cells also retain the immunologic markers characteristic of histiocytes, showing receptors for cytophilic antibody (72).

In Hodgkin's disease, application of these methods of identification requires a somewhat different approach. Most of the non-Hodgkin's malignant lymphomas consist of a relatively homogeneous population of neoplastic cells, but in Hodgkin's disease there is a population of neoplastic cells (the Reed-Sternberg cells and their mononuclear variants) admixed with a population that is presumed to be reactive—lymphocytes, plasma cells, and eosinophils, We have little direct information about the nature of the Reed-Sternberg cell. However, much indirect evidence suggests that Hodgkin's disease involves the thymic-dependent portion of the immune system. Early in the course of the disease, patients often manifest defects in cell-mediated immunity (73). This disease is also seen to involve



igure 3. Immunologist's view of the rganization of the immune system.

preferentially the thymic-dependent portions of the lymphoreticular system (67). We (74) and others (75) have also shown an augmented T-cell population in the tumorous lesions of Hodgkin's disease.

There seems to be an important interaction between the malignant cells (the Reed-Sternberg cells) and the admixed normal appearing lymphocytes. In suspensions prepared from Hodgkin's tissue, the small lymphocytes are often seen to be tightly adherent to the malignant cells (76). Furthermore, these adherent cells form E rosettes, indicating that they are in fact T lymphocytes (74, 77). This lymphocyte-Reed-Sternberg cell interaction can also be identified by scanning electron microscopy (74). The nature of this interaction is not as yet understood, but it is presumably of great importance in the pathophysiology of this disease.

I have shown briefly how the functional and anatomic components of the immune system can be manifested as neoplasia. We hope that this sort of approach will help us to understand the pathophysiology of the malignant lymphomas and will shed light on potential etiologies or therapies of this group of tumors.

Dr. Costan W. Berard: Doctor Jaffe has shown how, using cell surface markers, it is possible to relate some lymphoreticular neoplasms to the normal anatomic and functional components of the immune system. The identification methods she has presented are, however, static techniques and provide only inferential information about the functional activities of the cells. Some tumors show gross evidence of function, for example, pancytopenia due to phagocytosis in malignant histiocytosis (histiocytic medullary reticulosis) or a hyperviscosity syndrome due to the monoclonal serum spike in Waldenström's macroglobulinemia. In many other of these tumors, however, the cells must have more subtle functional activities. Doctor Green will discuss some of the current studies directed at elucidating the functional capacities and characteristics of the cells in this group of neoplasms.

Cellular Functions and Characteristics

Dr. Ira Green*: Before I discuss the functional aspects of these neoplastic cells, I would like to distinguish two separate types of studies. One type is done on the peripheral blood cells of patients with diseases, for example, Hodgkin's disease, in which the circulating cells are usually considered to be non-neoplastic. Defects can be ascertained in these cells by phytohemagglutinin stimulation for example, but such studies yield no direct information about the malignant cells.

The second type of study, which I shall discuss, is concerned with the malignant cells themselves. This includes studies in patients with diseases such as chronic lymphocytic leukemia or Sézary's syndrome. In both cases the vast majority of cells in the peripheral blood are malignant.

Before reviewing the studies in these patients, I shall first present the immunologist's view of the immune system (Figure 3). Although this is a functional organization of the immune system rather than the anatomic one described by Dr. Jaffe, it should be remembered that the interconnections and interactions between the various cells shown in the functional diagram occur within the architecture of the lymph node and that we do not fully understand the relation of the architecture of the lymph node to many of these collaborative interactions. As Dr. Jaffe noted, several types of cells are involved. Some differentiate in the thymus to form the T lymphocytes; others become the macrophages or monocytes; and still others are the B lymphocytes, which are the precursors of plasma cells. All these cells, together with antigen, go through a very elaborate dance in which various mediators are produced and special functions are performed by the various T lymphocytes: killer cells, cells that collaborate

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Table 5. T-Cell Functions end Lymphokines

T-Cell Functions	Soluble Mediators of Cellular Immunity (Lymphokines)
Mixed lymphocyte reactions	Macrophage migration inhibitory factor
Killer cells	Skin reactive factor
Helper cells for antibody formation	Leukocyte inhihitory factor
Amplifier cells	Lymphotoxin
Suppressor cell?	Osteoclast activation factor
Site for specific recognition of the carrier portion of the antigen	Chemotactic factor; blastogenic factor; interferon; transfer factor; lymph node pernieability factor

with the cells that produce antibody, and cells that produce lymphokinins of various types that activate macrophages (78).

Table 5 outlines some of the known functions of T lymphocytes and some of the mediators produced by immunologically competent cells. The molecular basis for ninny of these functions is not understood, and the function? are dcfinecl by a variety of in-vitro assays. There are cells, for example, that kill various kinds of target cells. Some cells engage in mixed lymphocyte reactions—that is, when one mixes together lymphocytes of two different persons, the lymphocytes undergo blast transformation. Some T cells interact with the B cells to produce antibody. Some cells suppress the function of others. Finally, T cells have receptors that recognize antigen and are presumably necessary for the initial aspects of antigen recognition. The nature of this receptor is still the subject of intense interest and controversy *(79).*

Some of the functions of these cells can be ascribed to a group of chemical substances called lymphokines (Table 5). At least 25 different substances have been described, and some have been carefully defined. For example, macrophage migration inhibitory factor has been sophisticatedly isolated and identified *(80).* Some of these other products have not been so carefully identified or defined molecularly; these mediators of the immune response are produced by T cells, and some of them are also produced by B cells or even by nonlymphoid cells (81).

Some of these mediators are produced by abnormal malignant cells. An example of the production of these mediators by such cells is seen in Sézary's syndrome, a chranic form of leukemia characterized hy marked erythroderma. Although this is a rare disease, we were able to study five patients during the past 2 years and examine some of the products made by their cells. It should be noted that the malignant cell in this particular disease is thymus-derived or a T lymphocyte (82, 83), whereas the lymphocyte of chronic lymphocytic leukemia is a B lymphocyte (84, 85).

Serum from these patients contains migration inhibitory factor for monocytes. This factor can also be found in vitro by direct away of the lymphocytes from these patients (86). The serums and cells from normal control subjects show no such activity. However, this activity has also been found in the serums of patients with various other lymphomas, including Hodgkin's disease (87).

Doctor Berard has alluded to the significance of the presence of this factor in the serums of these patients and to the fact that it is manufactured by the malignant cells. It is conceivable that the erythroderma in these patients is due to abnormal production of this material by the cells, causing the attraction of a large number of monocytes to the areas of erythroderma. However, whether this is the actual cause of the erythroderma or whether there is another mediator secreted or another mechanism responsible is not certain.

Another test commonly used for a variety of studies is the so-called mixed lymphocyte reaction (88). An important test for determining the degree of antigenic similarity between different individuals, it is widely used, for example, to predict the outcome of renal or other kinds of tissue allografting (89). The results of such tests correlate well with the survival of the allografts. The mixed lymphocyte reaction is also used to measure T-cell competence. The reaction is mediated principally hy thymus derived lymphocytes. When the lymphocytes of two normal individuals are mixed together, they undergo a marked proliferation, as measured by ^{3}H-thymidine incorporation. Lymphocytes from patients with Sézary's syndrome are in general markedly deficient in their ability to respond in the mixed lymphocyte reaction *(82).*

Also important is the fact that the neoplastic lymphocytes of one of the patients with Sézary's syndronte failed to act as stimulating cells when mixed with the cells of a normal subject. This is especially interesting because the patient's neoplastic cells could be typed with HL-A typing serum and had a normal complement of HLA antigens. At one time the HL-A antigens were thought to be the antigens recognized and necessary for this type of allogeneic stimulation to occur. This study, however, indicates that it is not the HL-A antigens themselves that are recognized and further supports evidence accumulated by independent genetic means to suggest that antigens determined by another closely linked locus are responsible for the mixed lymphocyte reaction *(90).* Thus, the use and study of homogeneous malignant cell populations can provide insights into the normal workings of the immune system.

Another key point is that although patients with Sézary's syndrome can be classified generally as having chronic T-cell leukemias with erythroderma, functional studies done in each patient show that each individual has a somewhat different spectrum of abnormality. Whether this fine analysis of functional defects in the cells of these patients will lead to information of clinical import remains to be seen.

Functional studies and the studies discussed by Dr. Gallo have an intriguing connection; there are four very provocative points. First, of the lymphoid cells, only the B lymphocytes have a receptor for the EB virus, which is thought to be the etiological agent of Burkitt's lymphoma and infectious mononucleosis (91). Neither T lymphocytes nor macrophages have this receptor. Furthermore, the Burkitt lymphoma cells themselves are a malignancy of the B lymphocyte *(92).* Is this a coincidence?

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Dr. Costan W. Berard: A
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Trends in Treatment

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Second, in the last 2 years a murine virus has been described, the Abelson virus, which after injection produces in mice malignancies with extremely short latent periods of about 30 days. The tumors that arise have a rather peculiar distribution in the skull and small bones, and all the tumors induced by this virus are either B-cell or plasma-cell tumors (93, 94).

A third finding of interest was reported by Olding, Jensen, and Oldstone (95). A certain virus (murine cytomegalovirus) can be used to infect murine cells and produce a latent viral infection. It causes no severe damage, and therefore the mouse appears normal. However, if the cells of these latently infected mice are activated by mixing together tissue from two different strains of mice, causing a mixed lymphocyte reaction, then the virus is activated. The tissue source for this activation experiment is crucial because if tissues containing thymus-derived lymphocytes are used to cause this allogeneic reaction, virus activation does not occur. Activation occurs only if the tissue source contains B cells. The authors suggest that this particular virus either is present only in B cells or can be activated only from B cells. These several observations in a sense bring together the virologic and immunologic spheres of information about lymphomas.

Table 6 summarizes some of these points. As Dr. Jaffe showed, the detection of markers on the surface of malignant lymphoid cells has helped to identify the nature of these cells and may lead to new advances in diagnosis and prognosis. I have discussed how these clonal populations of cells can be used to study the function of normal lymphocytes. Such studies may also provide clues to the pathophysiology of the various disease states, for example, the itching of patients with Hodgkin's disease or the erythroderma of patients with Sézary's syndrome. Another point, to which I just alluded, is the very provocative connection between virology and the receptors on the surface of particular lymphocytes for certain viruses. Finally, we hope that sometime in the future we may use these receptors or the idiotypic immunoglobulins on the surface of some of the cells, for example, in nodular lymphomas and in chronic lymphocytic leukemia (96), as highly specialized targets for certain types of immunotherapeutic attack.

Dr. Costan W. Berard: Although the new discoveries concerning reticuloendothelial neoplasms covered by the first three discussants have emanated primarily from laboratory research, equally impressive advances have been achieved in clinical oncology. Doctor DeVita will present recent trends in the therapy of some of these diseases.

Trends in Treatment

Dr. Vincent T. DeVita, Jr.*: I shall describe briefly what I think are the trends in the treatment of four important diseases: acute lymphocytic leukemia of childhood, acute myelogenous leukemia of adults, Hodgkin's disease, and diffuse histiocytic lymphoma.

The importance of the leukemias and lymphomas should not be underestimated. In 1975 we anticipate 30 000 new

* Director, Division of Cancer Treatment, National Cancer Institute.

Table 6. Significance of Functional Studies

New methods of classification of lymphomas
Aid in diagnosis, prognosis, and follow-up of effects of therapy
Clonal populations of cells
Identify products made by these cells, analysis of cell surface structure—provide clues as to function of normal cells
Use in preparing specific antibodies
Use in immunologic studies such as mixed lymphocyte reaction and cell-mediated lysis
Pathophysiology of disease may be explained by overproduction of lymphokines by malignant lymphocytes. for exampk, the erythroderma of Sézary's syndrome
Surface receptors could also be receptors for virus—only B cells have receptors for Epstein-Barr virus
Receptors or idiotypic immunoglobulins. or both, could be the target for highly specialized chemoimmune therapeutic attack

cases of lymphoma (97). Furthermore, because these diseases affect a young population, their impact in terms of person-years lost in general or from the work force assumes a greater importance than most other, even more common, malignancies. Actually, in the aggregate, the leukemias and lymphomas stand consistently fourth in morbidity and mortality rates in this country, behind such common killers as breast, lung, and colon cancer.

ACUTE LYMPHOCYTIC LEUKEMIA OF CHILDHOOD

It is now safe to say that we can cure a significant number of patients with acute lymphocytic leukemia of childhood. Survival is significantly greater among patients who receive the more aggressive modern treatment programs. Survival rates began to improve in the 1950s with the use of corticosteroids and the antimetabolite methotrexate. The impact of treatment programs was first felt with some intensity in the 1960s when combination chemotherapy programs came into force. Some of the early successful programs were initiated here at the National Cancer Institute. It is important to emphasize that 25 years ago, in 1950, the median survival for patients with acute lymphocytic leukemia was 2% months. Almost all patients were dead within 1 year of diagnosis. Now, as 5-year survivals approach 50% in some studies, median survivals hover between 3 and 5 years (98). The increased survival has stemmed from the availability of many chemotherapeutic agents, each with independent activity, that could be used in combination), such as the four-drug VAMP and POMP programs (vincristine, methotrexate, 6-mercaptopurine and prednisone) introduced at the National Cancer Institute in the early 1960s by Dr. Freireich. Combination chemotherapy is now used to induce complete remissions (achievable in 95% of patients with little or no mortality) and for prolonged treatment after remission is achieved (consolidation therapy) to prevent relapse. With consolidation therapy, the duration of time a patient remains free of disease after all treatment is stopped has increased. Commensurate improvement in survival after cessation of therapy has been clearly shown. The use of the word "cure" is based on the flattening of the survival curves 5 years after treatment.

When it was realized that the central nervous system is often involved in relapse in patients in bone marrow

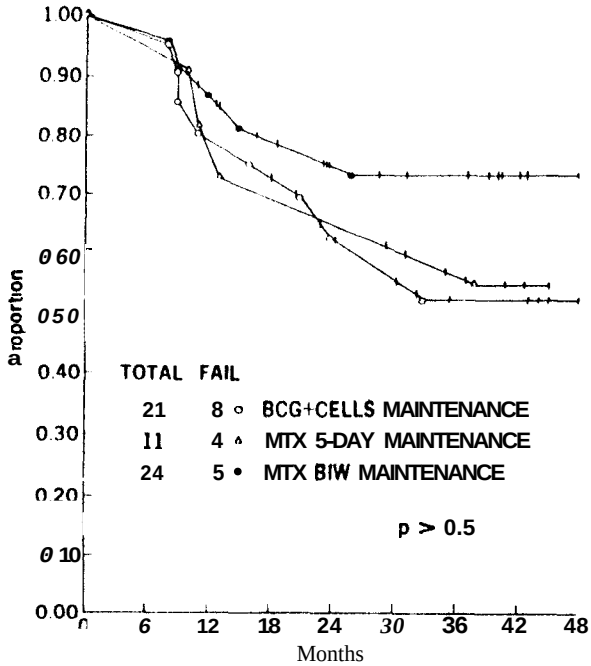

Figure 4. Survival of patients with acute lymphocytic leukemia in the National Cancer Institute series. MTX = methotrexate.

remission, the concept of total therapy emerged. Data from St. Jude's Hospital in Memphis, Tennessee, show the results of aggressive remission induction treatment, long consolidation therapy (in the studies cited for 2 to 3 years), and irradiation of the central nervous system plus the use of intrathecal niethotrcxate (99). The survival of patients followed in recent studies can be extrapolated to the T-year point, and about half of these patients remain free of disease Similar results have been achieved by the members of acute leukemia group B* and at the National Cancer Institute (100). The long-term, disease-free survival of patients in the National Cancer Jnstitute study is shown in Figure 4. The flat tails on the survival curves indicate a decreasing rate of relapse and risk of mortality after the first 2 years. Today, with 10-year follow-up data from older studies, we estimate that patients who are free of disease 5 years after intensive treatment have only about a 10% risk of developing recurrent leukemia. Furthermore, we can now segregate patients with acute lymphocytic leukemia into prognostic categories based on cell-surface markers. Patients with "null cell" disease have an excellent prognosis, whereas those whose lymphoblasts are identifiable as T cells, particularly those presenting with mediartinal tumors, have the worst prognosis. Future therapy will undoubtedly be tailored more and more to the aggressiveness of the disease, based in part on these cellular markers.

Acute lymphocytic leukemia cells seem to be antigenic. One would expect immunotherapy to be successful in treating patients with this disease, particularly as a form of remission maintenance therapy after the number of cancer cells has been diminished by remission induction treatment with combination chemotherapy. In 1969, Mathé and colleagues (101) did, in fact, administer intensive cytoreductive chemotherapy followed by bacillus Calmette-Guerin (BCG) (and in many cases allogeneic leukemia cells as a vaccine) to a group of patients with acute lymphocytic leukemia. All of the patients in the control population relapsed by 130 days, whereas some of the patients in the group receiving immunotherapy remained free of disease for prolonged periods. The results of this study are somewhat clouded because chemotherapy varied from patient to patient and the study was not strictly controlled. Later studies (102, 103) using immunotherapy for remission maintenance in patients with acute lymphocytic leukemia have not as yet confirmed Mathé's results. The Concord study in England, which used a different BCG preparation and a different method of administration (heal gun as opposed to scarification), did not show any positive effect of immunotherapy as a remission maintainer. However, remission induction therapy was less intense than that used by Mathé, and there were many other differences. The study by the National Cancer Institute's Pediatric Oncology Branch (100) also reported no advantage from the addition of BCG to the maintenance program (Figure 4). Despite these negative results, the data are as yet insufficient to conclude that immunotherapy is of no value in the treatment of acute lymphocytic leukemia.

Where do we go from here in acute lymphocytic leukemia of childhood? One need not improve much on the 95% rate of remission induction now achievable with modern combination-drug programs. Obviously, with better drugs and more intensive combinations, we should be able to treat subclinical disease more effectively, to improve the disease-free survival. Also, it would be a great advantage to achieve these results with less toxicity. Current treatment programs are being designed with these points in mind, particularly with reference to the influence of cell markers on prognosis. What we need most of all is an assay for residual tumor-cell populations of less than 10^8 cells, so we can accurately determine how long to treat the patient. Is one year enough? Two years? Three years? Therapy wears on the patient and the family after a while, and it would be extremely useful to be able to detect accurately small amounts of residual disease, or the absence of it, to guide the termination of therapy.

ACUTE MYELOGENOUS LEUKEMIA

The trends in acute myelogenous leukemia have been the same as those in acute lymphocytic leukemia; that is, treatment programs emphasize intensive remission induction, consolidation, and immunotherapy, with the following exceptions: there are fewer active drugs; the disease affects an older age group; there is a 30% mortality rate associated with induction of remission; and even the most effective combinations have only recently been able to yield complete remission rates in excess of 50% (and thus improve median survivals) (104, 105). The complete re-

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mi rate is closely related to the age of the patients, dropping to about 25% in patients over age 50 (106). An argument has raged in the literature for many years about whether or not to treat acute myelogenous leukemia. I personally find the discussion somewhat specious, because those patients who do achieve a complete remission, even with older treatment programs, do in fact have increased survival free of disease. Those who do not respond to therapy do not live a shorter period of time than those who not treated at all. The two most practical and effective drug treatment programs now available for the treatment of acute myelogenous leukemia are the combination of arabinosyl cytosine and 6-thioguanine reported by Gee, Yu, and Clarkson (104) and the four-drug COAP program reported by Whitecar and colleagues (see Reference 105). In both series complete remissions were regularly achievable in more than 50% of patients.

Immunotherapy seems to be playing a greater role in acute myelogenous leukemia than in acute lymphocytic leukemia. This may tie in with some of the information presented earlier by Dr. Gallo, particularly the identification of specific proteins on the surface of acute myelogenous leukemia cells, which may be related to a viral origin and may account for the apparently greater immunogenicity of these cells.

In a study from St. Bartholomew's Hospital in London that used BCG plus allogeneic cells after induction of remission with arabinosyl cytosine and daunorubicyn (107), and in two other studies, one using BCG and cells and another using BCG alone, prolongation of remission favoring the use of immunotherapy has been shown (108, 109). The study with BCG alone suggests that the addition of cells may not be necessary to achieve the desired effect. A similar study using immunotherapy after a different three-drug combination has been reported from the M. D. Anderson Hospital in Houston (110). This study also shows that patients receiving immunotherapy have a longer remission duration than controls.

Bodey and colleagues (111) recently introduced the concept of late intensification therapy in acute myelogenous leukemia. This approach uses retreatment with the aggressive remission induction potocols in patients who are in remission and are well 1 year after initial treatment. This type of treatment is easy to use in such patients whose marrow is normal at the time, and the early results are provocative. This approach warrants further consideration in acute myelogenous leukemia and in other diseases whete high remission rates are achieved but the relapse rate is significant.

Where do we go from here in acute myelogenous leukemia? We need better drugs for remission induction and better methods to avoid the large loss of life during induction treatment. The mortality remains prohibitively high. Supportive care programs using sterile environments and leukocyte and platelet transfusions have been helpful, but they have not been good enough in and of themselves to improve survival and probably will not be without newer treatment programs. Of great interest is the prospect of detecting minimal disease by using immunologic assays of specific marker proteins derived from the cell surface, or of enhancing the specificity of immunotherapy by using the same approach based on the work presented by Dr. Galla.

HODGKIN'S DISEASE

The results of treatment of this disease have been the most dramatic of perhaps any tumor studied in the past decade. Current data suggest that 70% of all patients with Hodgkin's disease who walk in the door of a center studying this disease should be able to achieve a normal life span with either radiotherapy, chemotherapy, or radiotherapy and chemotherapy combined. The improved results can be traced to a better understanding of the pathology and natural history of the disease through staging laparotomy, technological advances in radiotherapy equipment and its use, and vastly improved chemotherapy program* (112).

It was recognized by Dr. Vera Peters in the early 1950s and by Dr. Eric Easson in the early 1960s that some patients with localized tumors treated with radiotherapy achieved normal life expectancy. Other radiotherapists, including Dr. Ralph Johnson at the National Cancer Institute and br. Henry Kaplan at Stanford University, rapidly expanded these observations. Doctor Kaplan analyzed, from reports in the literature, the recurrence rate in treated fields, and he emphasized the marked dose-response effect between radiation dose and the ability to sterilize involved lymph node areas. He showed that patients seldom develop recurrences if the dose to a field is more than 4000 reds (113). These observations and the realization that such doses could be safely delivered to large volumes of lymph node-bearing areas led physicians at both Stanford University and the National Cancer Institute to use what is now referred to as total nodal irradiation. At the National Cancer Institute, Johnson (114) randomized patients between the less aggressive extended-field radiotherapy and total nodal irradiation. The latter treatment proved superior in all patients except a subpopulation with nodular sclerosing Hodgkin's disease, in which both treatments were equivalent. Overall, total nodal irradiation has proved superior to extended-field radiotherapy in patients staged without routine use of laparotomy and before the realization that today's more effective chemotherapy programs may make less aggressive radiotherapy more acceptable, because they can save those patients in whom disease recurs after radiotherapy alone.

Our own contribution to this area was the development of the MOPP (nitrogen mustard, vincristine, procarbszine, and prednisone) four-drug combination chemotherapy program, which increased the complete remission rate from 20% to 80% and markedly lengthened the duration of remission (from a median of 2 months to a median of 42 months) and survival of patients with advanced Hodgkin's disease (115). Before MOPP therapy the median survival of patients with stage III and IV Hodgkin's disease was consistently 2 years or less. Less then 10% of patients survived, and even fewer were free of disease after 5 years. In contrast, treatment with MOPP improved the 5-year survival to 70% (116). The survival curve of patients treated at the National Cancer Institute has now reached

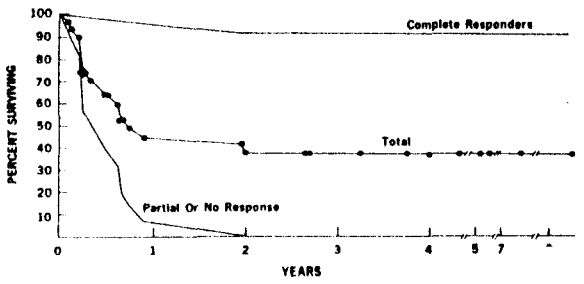

Figure 5. Survival of patients with advanced diffuse histiocytic lymphoma in the National Cancer Institute series

10 years. The diseuse-jree *survival* for the entire group of patients with stage III or IV disease who achieved complete remiscion is **50%** at **10** years. It is crucial to examine these results by stage and histology. More than 90% of patients with stage IIIA and IVA Hodgkin's disease had complete remissions, and, to date in our study, only one patient of those with stage IIIA and IVA disease has relapsed. Although patients in similar stages respond well to radiotherapy, the remarkable results of MOPP therapy would be difficult to improve on using radiotherapy or other combinations of drugs. The results are not as good in patients with stages IIIB and IVB disease, in whom disease-free survivals range from **25%** to **40%** depending on the histologic type. The variation in results by stage. the presence or absence of symptoms, and the histologic type has caused some confusion in the interpretation of the literature. In contrast to the results with radiation therapy, nodular sclerosis histology adversely affects the disease-free survival rate in patients treated with MOPP.

Thew results have been confirmed in a report from the Southwestern Cooperative Group (**117**) studying the effect? of maintenance MOPP treatment after remission induction This group showed a complete remiscion rate equ ralent to the results from the National Cancer Institute, and a slight advantage for maintenance therapy with MOPP, when compared with the control group, in increasing the number of patients remaining in complete remission **A** study from the National Cancer Institute (118). examining the same question but using a more intensive and prolonged induction period than in the Southwestern Group study, did not show a beneficial effect of maintenance treatment with either MOPP or BCNU [1,3 bis (2-chloroethyl)-1-nitrosourea] when compared with a control population receiving no drug maintenance treatment. This question is still unresolved. **A** study from St. Bartholomew's Hospital in London, using a modified MOPP program in which vinblastine is substituted for vincristine, has shown similar results (**119**).

Where do we go from here in Hodgkin's disease? There are three trends. One is to combine irradiation therapy and drugs, attempting to use lesser amounts of irradiation than usually indicated in a given stage. Another trend is to develop a drug combination, not cross resistant with the MOPP program, to prevent relapse in patients who have a high risk of relapse after achieving a complete remission with MOPP. **A** third approach is to use immunotherapy with or without chemotherapy as a form ...te-mission maintenance. Studies now in progress in several institutions are examining all these questions. In a dy at Stanford, patients with stages I, II, and IIE disease and favorable histology (lymphocyte predominant and nodular sclerosis) are given mantle or involved-field radiation therapy with six cycles of MOPP chemotherapy, and the results are compared with those of a group receiving only total nodal irradiation therapy. Patients with unfavorable histology are given total nodal irradiation both cases, but one group receives six cycles of MOPP as well. These studies should answer some very important questions, and the Anal results are eagerly awaited risk of carcinogenesis in using two such programs should dampen the enthusiasm to use such approaches routinely until enough data support them.

In trying to develop a drug combination that is not cross resistant Io MOPP, Bonadonna and co-workers (**120**) used four drugs (adriamycin, bleomycin, vinblastine, and the new imidazole derivative, dacarbazine), each individually active, to treat Hodgkin's disease and compared his program with MOPP. The early results show a complete remission rate equivalent to MOPP. Furthermore, the early results also show that patients who fail on one program may benefit from the alternate treatment, suggesting that the two used in tandem might be effective in preventing relapses. Although other programs offering modifications of MOPP—substituting a nitrosourea for nitrogen mustard or adding bleomycin—or combinations using different schedules of the same drugs used in MOPP have shown equivalent or even slightly better complete remission rates, none has as yet produced equivalent disease-free survivals ("cures"), and none fulfills the requirement of being non-cross resistant to MOPP (**121**). The preliminary data thus far available on immunotherapy (**122**) are not yet sufficient to interpret accurately the role of immunotherapy in this disease.

Fifteen years ago Hodgkin's disease was treated by radiation therapy for cure and by ineffective chemotherapy for palliation, and even effective palliation was questioned The development of a single effective drug program, MOPP, improved the lot of the patients with advanced disease who, at that time, did not usually receive effective treatment with radiotherapy. In addition, it gave researchers a neatly packaged therapeutic tool to balance with and against radiotherapy. The advantage of the MOPP program is that it is now an old friend whose therapeutic results and side effects are predictable. The emphasis in combined modality studies can now be on the influence of the two treatments. irradiation and chemotherapy, on the natural history of the disease and not on an analysis of the details of the treatment program itself. MOPP produces a respectable cure rate and is therefore the logical drug program to use in combined modality trials, as in the Stanford study referred to above. It remains the drug treatment of choice for stages III and IV disease until other programs, now under study, show equivalent disease-free survivals beyond the high-risk point of the survival curve (*5* years) after all treatment stopped, or unless other regimens producing equivalent

results are significantly easier to use.

DIFFUSE HISTIOCYTIC LYMPHOMA

I hope that the pathologists will tell us the true cell(s) of origin (histiocytes, reticulum cells, null cells, or maybe B cells) and whether this is one or many diseases. Using a modified MOPP program in which cyclophosphamide is substituted for nitrogen mustard (C-MOPP), we have achieved a 41% complete remission rate in patients with stages III and IV disease (122). The point worthy of repeated emphasis is that only one of the patients who achieved a complete remission has relapsed; the rest remain free of disease. None have been followed less than 3 years, and the longest survivor, now past 10 years from the end of treatment, remains free of disease (Figure 5). As shown in Figure 5, everything that transpires in this disease does so within 2 years. Disease-free survival beyond 2 years, regardless of the initial stage of the disease or the type of treatment, is tantamount to cure. The only difference in survival curves generated using radiotherapy for patients with localized disease (stage II) and modern chemotherapy programs for patients with advanced disease (stages III and IV) is that the median survival of patients with advanced disease is 6 months shorter. Similar results have now been achieved by the group at Yale (123) using another drug combination.

An important aspect of the natural history of diffuse histiocytic lymphoma is that many patients present with what seems to be localized disease. However, the studies from Stanford in which Jones and associates (124) used involved-field, extended-field, or total nodal irradiation therapy show that patients with apparently localized disease (especially stage II) do not achieve good disease-free survival (less than 30%) with radiotherapy alone no matter how aggressive the treatment. Staging laparotomy has not been helpful in this disease. Disease outside the known area of tumor involvement has been detected by surgery in only a small number of patients undergoing this procedure (125); and yet the relapse rate of such patients treated with local or extensive radiotherapy is very high. Since results with combined drug programs in patients with advanced disease are as good as or better than results with radiotherapy in localized disease, we have initiated studies ucing involved-field radiation therapy plus C-MOPP or C-MOPP alone in patients with stage II disc - Newer and, we hope, more effective drug combinations are being developed for patients with stages III and IV disease.

In summary, dramatic advances have been made during the past 10 years in our ability to control the diseases discussed above. We should not lose sight of another very important fact about the lyniphomas and leukemias. They have served as a stalking horse for the treatment of other tumors. The enthusiasm created by the use of combined modalities in diseases such as Hodgkin's disease, which required close cooperation among three separate specialties—radiotherapy, surgery. and medical oncology—has led us to apply the same principles to the management of other more common tumors, in some cases with considerable success.

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