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RECENT DEVELOPMENTS IN THE NON-HODGKINS LYMPHOMAS

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The combined incidence of all lymphomas ranked 7th among cancers in 1984. The incidence of lymphoma may be gradually increasing; 42,000 new cases were counted in 1986 (1). About 3/4 of these tumors are non-Hodgkin's lymphomas. As with several other cancers, the age specific incidence rises steeply after age 50, reaching 110 per 100,000 population in the 9th decade (2). However, the average age of onset is substantially lower than that of common epithelial cancers, and the economic impact of this disease in terms of person years of life lost is substantial. Since a major risk factor is immunosuppression (3), the numbers of new cases in younger individuals is expected to increase yearly as the epidemic of acquired immune deficiency syndrome (AIDS) peaks.

Since about 3/4 of adult lymphomas are derived from lymphocytes in the B lineage (4), this selective review will focus upon B-cell lymphomas. In this Grand Rounds I will review recent developments in etiology, classification, detection, staging and treatment of these lymphomas. The phenomenal growth in knowledge of B lymphocyte growth and development is now shaping the design of clinical trials.

Etiologic Clues

It has long been known that immunodeficiency states are a risk factor for non-Hodgkins lymphoma (3). The high incidence of B-cell lymphomas in AIDS has reemphasized this relationship. The spectrum of lymphomas observed in AIDS patients is summarized in **(Table 1)** (5).

Table 1. Histologic Subtypes of Non-Hodgkin's Lymphomas in 90 Homosexual Men.

Histologic Subtype	No. of Patients
High-grade malignant lymphoma	
Small, noncleaved cell	32
Large cell, immunoblastic	22
Lymphoblastic	2
Total	56 (62%)
Intermediate-grade malignant lymphoma	
Diffuse, large cell	17
Diffuse, small cleaved cell	8
Diffuse, mixed	1
Total	26 (29%)
Low-grade malignant lymphoma	
Small lymphocytic, plasmacytoid	3
Follicular, small cleaved cell	3
Total	6 (7%)
Miscellaneous	
Unclassified	2 (2%)

(From Reference 5)

Note that the majority of these tumors are high grade lymphomas of small noncleaved and immunoblastic histologies, and that involvement of extranodal sites such as the CNS, marrow and GI tract is very common. (**Table 2**)

Extranodal Sites of Non-Hodgkin's Lymphoma in 88 Patients.

Site	No. of Patients *
Central nervous system	
Brain mass	21
Meninges	14
Cranial/peripheral nerves	5
Paraspinal	5
Total	38
Bone marrow	30
Skin/mucosa	
Intraoral	4
Anorectal	3
Thigh	3
Popliteal fossa	1
Ear lobes	1
Cutaneous nodules	1
Scalp	1
Total	14
Bowel	15
Lung	8
Liver	8
Kidney	2
Orbit	2
Pericardium	1
Bone	1
Gallbladder	1

*Some patients had lymphoma at more than one site.

(From Reference 5)

About 50% of these AIDS related lymphomas are associated with the Epstein-Barr virus (EBV) (6). In biopsies of nodes from patients with generalized lymphadenopathy, Dalla Favera and coworkers find oligoclonal patterns of immunoglobulin gene rearrangements (7). These patterns suggest that a relatively small number of B cells are gaining clonal dominance in these nodes. In patients with frank lymphoma, one of these clones develops a characteristic chromosomal translocation involving the c-myc locus on chromosome 8 and the immunoglobulin heavy chain gene locus on chromosome 14 (t(8;14)(q24;q32) (7). The hypothesis is that either the EBV virus or some other influence promotes unregulated proliferation of B-cell clones in the setting of disordered immune regulation. This proliferation increases the likelihood of secondary genetic errors. One of these mistakes fuses the c-myc and immunoglobulin loci, and this translocation is selected for because it provides a powerful growth advantage to that particular clone. Dalla Favera's group has shown directly that enhanced expression of the

c-myc gene in human B-cell clones leads to enhanced growth potential as measured by increased cloning ability, growth in reduced serum concentration, tumorgenicity in nude mice and loss of cell adhesion (8).

The development of lymphoma in AIDS patients is analogous to tumor progression in African children with Burkitt's lymphoma. In the latter case, immunosuppression is thought to arise from malaria and other chronic infections which, along with EBV, lead to polyclonal B-cell stimulation. In this setting, the same chromosomal translocations lead to high grade lymphomas because of the selective growth advantage provided by activation of the c-myc oncogene. Multiple clones of proliferating B-cells have been associated with other states of immunosuppression, including allogeneic cardiac (9) and bone marrow (10) transplantation.

Interesting epidemiological observations in Sweden (11), the United States (12) and in New Zealand (13) have suggested that chemical carcinogenesis may be important in the development of lymphoma. These studies have demonstrated an increased risk of non-Hodgkin's lymphoma in agriculture workers. In Sweden and the United States, this risk was associated with exposure to herbicides such as 2,4 dichlorophenoxyacetic acid (**Table 3**).

Table 3.—Non-Hodgkin's Lymphoma in Relation to Duration, Frequency, and Latency of 2,4-Dichlorophenoxyacetic Acid Use

	No. of Cases	No. of Controls	Odds Ratio (95% Confidence Interval)
Never farmed	37	286	1.0
Duration of use,* y			
1-5	3	16	1.3 (0.3, 5.1)
6-15	7	22	2.5 (0.9, 6.8)
16-25	8	15	3.9 (1.4, 10.9)
≥26	6	17	2.3 (0.7, 6.8)
χ for trend	3.560	. . .	. . .
P (one-tailed)	.0002	. . .	. . .
Frequency of use,† d/y			
1-2	6	17	2.7 (0.9, 8.1)
3-5	4	16	1.6 (0.4, 5.7)
6-10	4	16	1.9 (0.5, 6.7)
11-20	4	9	3.0 (0.7, 11.8)
≥21	5	6	7.6 (1.8, 32.3)
χ for trend	3.733	. . .	. . .
P (one-tailed)	.0001	. . .	. . .
First year of use‡			
1966 or later	5	21	1.9 (0.6, 6.0)
1956-1965	9	23	2.9 (1.1, 7.2)
1946-1955	8	24	2.1 (0.8, 5.6)
Before 1946	2	2	6.2 (0.6, 65.3)
χ for trend	3.561	. . .	. . .
P (one-tailed)	.0002	. . .	. . .

*Five controls had missing data.
†One patient and ten controls had unknown frequency of exposure.
‡First available for use in 1942.

(From Reference 12)

In New Zealand, on the other hand, risk was associated with fencing work (contact with dihydrated arsenic pentoxide) and slaughterhouses (exposure to 2,4,6-trichlorophenol) (13). Therefore, the possibility that chlorinated hydrocarbon herbicides and arsenic compounds are chemical lymphomogens deserves serious consideration and further study.

Genes that are potentially important in the development of human lymphoma have been identified by systematic analysis of non-random chromosomal translocations associated with specific histologic subtypes of lymphoma. At least some of these translocations fuse expressed genes with immunoglobulin loci which are of course transcriptionally active in B-cells. Thus, the control of expression these genes is brought under the influence of immunoglobulin gene regulatory elements. I have already mentioned the t(8;14) translocations associated with high grade B-cell lymphomas including small noncleaved and immunoblastic subtypes (**Figure 1**).

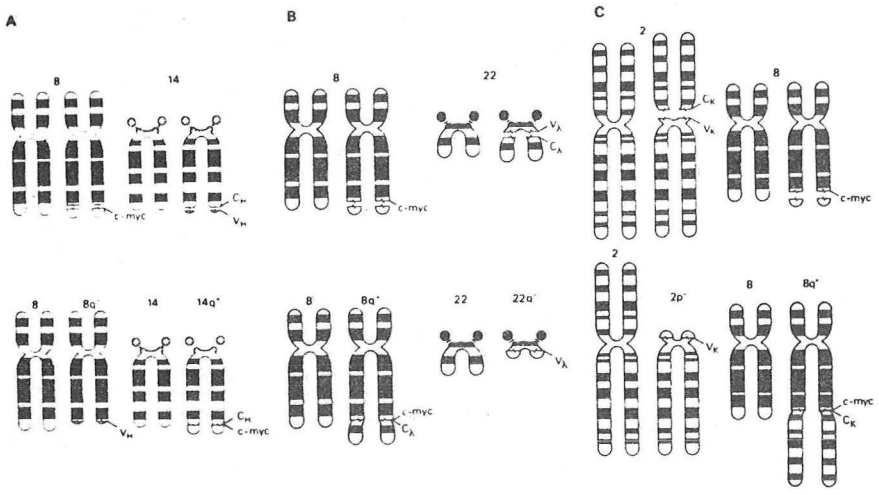
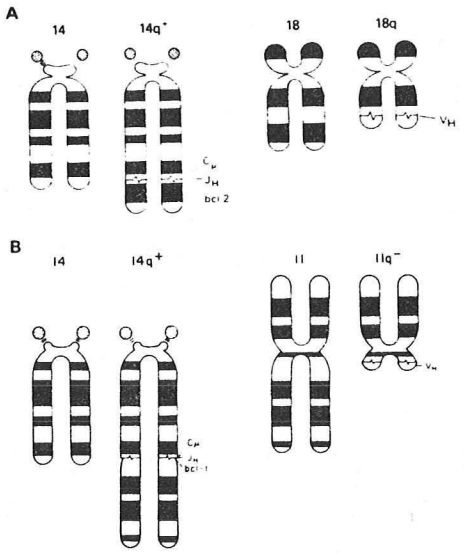


Fig 1. In Burkitt's lymphomas with the t(8;14) translocation, the c-myc oncogene translocates to the heavy chain locus (A), and a portion of the immunoglobulin locus (V_H) is translocated to chromosome 8. In Burkitt's lymphomas with the less frequent t(8;22) (B) and t(2;8) (C) translocations, the c-myc oncogene remains on the involved chromosome 8, but the genes for the immunoglobulin light chain constant regions (C_κ and C_λ) translocate to a region 3' (distal) to the c-myc oncogene on the involved chromosome 8 ($8q^+$). Again with these translocations, the immunoglobulin loci are split so that sequences that encode for the variable portion of the immunoglobulin molecule (V_κ or V_λ) remain on chromosome 2 or 22, respectively.

(From Reference 14)

The oncogene involved in these cases is c-myc. Rearrangement of another gene, bcl-2, as been found in at least 60% of follicular lymphomas (**Figure 2**).



Chromosome translocations in B cell lymphomas and leukemias of adults. In follicular lymphomas with the t(14;18) translocation, the bcl-2 gene, normally located on band q21 of chromosome 18, translocates to the heavy chain locus on chromosome 14 (A). In lymphomas and leukemias with the t(11;14) translocation, the bcl-1 gene, normally located on band q13 of chromosome 11, translocates to the heavy chain locus on chromosome 14 (B). (From Reference 14)

This is the most common type of B-cell lymphoma in the adult. The bcl-2 locus is normally found on chromosome 18; it is rearranged into the immunoglobulin heavy chain gene locus on chromosome 14 in most follicular lymphoma (15). The normal function of this gene is not known. It is transcribed actively in pre-B lymphocytes, to a lesser extent in activated B-cells and minimally in other types of cells. Thus, the bcl-2 gene encodes a stage specific, B lineage specific product whose derangement is an important step in the development of follicular lymphoma. Interestingly, the location of these break points immediately upstream of the immunoglobulin heavy chain J_h locus strongly suggests that this translocation is a consequence of a genetic error which happens at a pre-B stage of development. Nonetheless, the tumor is an expansion of mature germinal center B-cells which are the progeny of this transformed pre-B cell. Note that the immunoglobulin heavy chain expressed by the lymphoma is encoded by the IgH gene on the chromosome 14 not involved in the translocation.

In summary, elucidation of the roles of the c-myc and bcl-2 genes in B-cell growth in differentiation are providing important clues

to the pathogenesis of many non-Hodgkin's lymphomas.

Recent Trends in Classification

Classification of non-Hodgkins lymphomas is a source of great confusion to clinicians. This confusion stems from major progress in both clinical oncology and immunobiology. Beginning in the 1960's, careful prospective studies of natural history were correlated with histologic patterns. In these studies, morphologic analysis stood on its own and predated advances in understanding of lymphocyte structure and function. Nonetheless, the Rappaport histologic classification (16) was shown to be a valuable guide to prediction of biologic behavior in of these neoplasms. An explosion of information on lymphocyte structure and function began in the latter half of the same decade. This knowledge opened the way to immunophenotypic analysis of lymphomas. Together with detailed morphologic analysis of germinal center lymphocytes, this information forced a revision in lymphoma classifications. For example, pathologists learned that virtually all lymphomas were derived from resting and/or activated lymphocytes rather than histiocytes. Unfortunately for the non-specialist, these developments led to a proliferation of classifications none of which immediately were correlated with natural history. Therefore the Rappaport classification has continued in use. However, in 1979 an international panel of pathologists proposed a new Working Formulation classification that permitted translation between the several competing schemes.

(Table 4).

A Working Formulation of Non-Hodgkin's Lymphoma for Clinical Use: Recommendations of an Expert International Panel; Comparisons to the Rappaport Scheme

	WORKING FORMULATION	RAPPAPORT TERMINOLOGY
LOW GRADE		
A.	Malignant lymphoma Small lymphocytic consistent with chronic lymphocytic leukemia plasmacytoid	Diffuse well-differentiated lymphocytic
B.	Malignant lymphoma, follicular, predominantly small cleaved cell diffuse areas sclerosis	Nodular poorly differentiated lymphocytic
C.	Malignant lymphoma, follicular mixed, small cleaved and large cell diffuse areas sclerosis	Nodular mixed lymphocytic histiocytic
INTERMEDIATE GRADE		
D.	Malignant lymphoma, follicular Predominantly large cell diffuse areas sclerosis	Nodular histiocytic
E.	Malignant lymphoma, diffuse small cleaved cell	Diffuse poorly differentiated lymphocytic
F.	Malignant lymphoma, diffuse mixed, small and large cell sclerosis epithelioid cell component	Diffuse mixed lymphocytic-histiocytic
G.	Malignant lymphoma, diffuse large cell cleaved cell non-cleaved cell sclerosis	Diffuse histiocytic
HIGH GRADE		
H.	Malignant lymphoma large cell, immunoblastic plasmacytoid clear cell polymorphous epithelioid cell component	Diffuse histiocytic
I.	Malignant lymphoma lymphoblastic convoluted cell non-convoluted cell	Diffuse lymphoblastic
J.	Malignant lymphoma small non-cleaved cell Burkitt's follicular areas	Diffuse undifferentiated

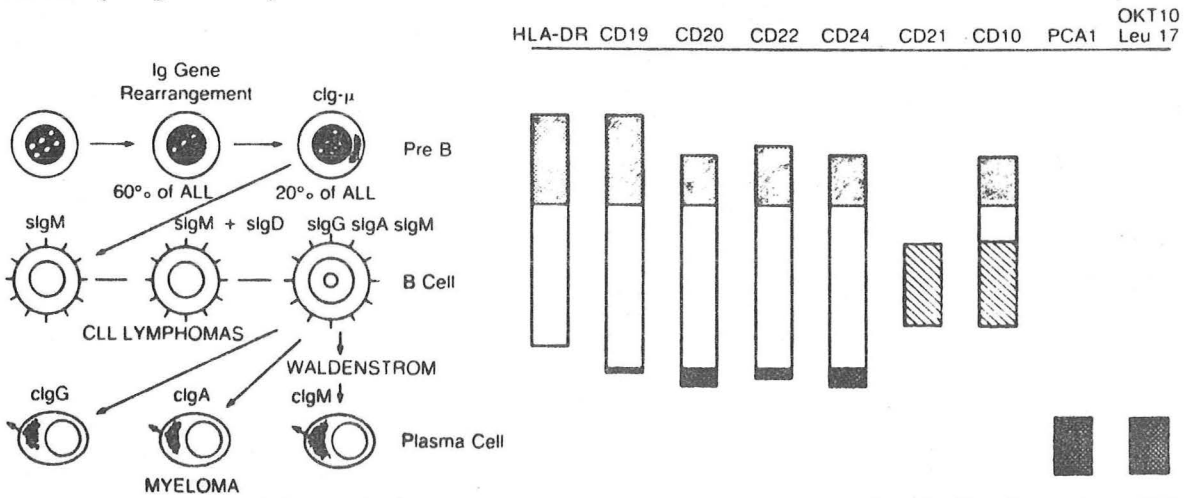
(From Reference 16a)

Incidentally, their work also showed that all of these schemes of classification were equally useful in predicting biologic behavior (17). The ten diagnostic entities of the Working Formulation are subgrouped into low grade, intermediate grade, and high grade lymphomas. Classification in all schemes is based upon overall pattern of neoplastic cells (follicular or diffuse) and cytology (small cell, large cell, mixed, cleaved or non-cleaved lymphocyte). The recent trend is to describe lymphomas in both Rappaport and Working Formulation terminology.

The Working Formulation is based on traditional morphologic assessment and does not rely upon immunophenotypic analysis. It is not possible in all cases to predict immunophenotype from histopathology. At the present time, major clinical decisions are based upon histopathologic analysis. Additional techniques of immunophenotypic, genetic and cytogenetic analysis are very helpful in confirming histopathologic impressions, particularly in borderline cases. For example, the reproduceability of pathologic diagnosis is low in certain categories. As I will discuss shortly, these newer techniques will become progressively more important as disease staging and treatment increasingly depend upon immunophenotype and genotype.

Immunophenotypic analysis has shown that about 3/4 of all non-Hodgkin's lymphoma in the adult are derived from B lineage cells. The key finding is the presence of idiotypically homogeneous immunoglobulin molecules on and/or in the tumor cells (18). This discovery not only defined the lineage of these lymphomas but also showed that the vast majority were monoclonal neoplasms (19). Futhermore, the idiotypic regions of these clonal markers provide unique targets for detection and therapy. I will discuss trials based upon this concept toward the end of the hour.

Many investigators have developed a host of additional B-cell differentiation markers defined by monoclonal antibodies (20). These markers define stages of maturation of normal B-cells and classify lymphomas malignancies according to stage of maturation arrest (**Figure 3**).

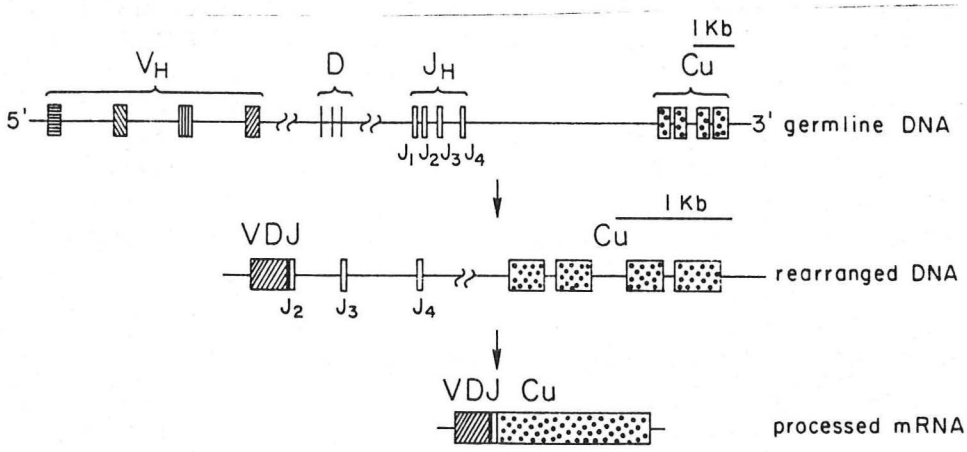


Schematic diagram correlating stages of normal B cell development with expression of selected cell surface antigens. Lightly shaded, unshaded, and darkly shaded areas correspond to precursor, mature, and terminally differentiated B cells, respectively. Diagonal lines indicate variable expression. (Reproduced with modification from: Jaffe ES, Cossman J: Immunodiagnosis of lymphoid and mononuclear phagocytic neoplasms. In: Rose NR, Friedman H, Fahey JL (eds): Manual of clinical laboratory immunology. 3rd ed. Washington, D.C.: American Society for Microbiology, 1986) Used with authors' permission. (From Reference 20)

As with immunoglobulin idiotype, cell surface differentiation markers such as CD20 are being exploited as targets for therapy with monoclonal antibodies.

One pan-T cell marker, CD5, revealed an interesting heterogeneity of B cell neoplasms. The small lymphocyte lymphoma/chronic lymphocytic leukemia group of tumors, which are CD5-positive and express low density surface immunoglobulin, are immunophenotypically as well as morphologically distinguishable from follicular lymphoma cells, which are CD5-negative and express high density membrane Ig (21). This CD5-positive subset of B-cell lymphomas stimulated a search for a normal B-cell counterpart. In fact, normal CD5-positive cells were found to be prevalent in fetal spleen and in bone marrow transplant recipients (22). Interestingly, this subset of normal B-cells secretes rheumatoid factor when stimulated <u>in vitro</u> (23). The occasional tendency of CLL to associate with autoimmune hemolytic anemia or thrombocytopenia may represent two manifestations of an underlying disorder of immunoregulation of the CD5-positive lymphocytes: one leading to pathological autoantibody formation and the other to neoplasia.

<u>Genetic methods</u> of analysis have also contributed to refined classification of non-Hodgkin's lymphoma. In the process of differentiation, B lymphocytes rearrange segments of immunoglobulin gene sequences to create a function variable region gene (**Figure 4**).



Rearrangement of heavy chain gene segments on chromosome 12 of the mouse. The scale on line one is different than that on lines two and three. The number and linkages of *V* genes within the *V* gene cluster and *D* genes within the *D* gene cluster are not known. C_μ denotes the constant gene for IgM, which is interrupted by three non-coding sequences. The joining of *V*, *D*, and *J* gene segments may occur by deleting the intervening DNA. Each *V* gene segment has a leader sequence that eventually is cleaved from the protein (not shown). (From Reference 23a)

These rearrangements distinguish B from other cells, a difference that can be demonstrated by restriction endonuclease digestion of tumor DNA followed by hybridization of the fragments with immunoglobulin gene probes. In non B-cells, a germ line configuration is seen representing unrearranged gene segments. In normal B-cell populations, there are so many different gene rearrangements reflecting polyclonal cell populations that no single rearranged restriction fragment stands out on the Southern blots. In a B-cell malignancy, the clonal rearrangement is found in a sufficiently large fraction of the total cells that a single rearranged band is evident in the analysis. This band suggests both B-lineage derivation and monoclonal proliferation. Taken together, these findings suggest but do not prove that a B-cell neoplasm is present. Immunoglobulin heavy chain gene rearrangements are seen in about 15-20% of phenotypic T-lineage and a few myeloid neoplasms. The presence of immunoglobulin light chain gene rearrangements is thought to provide a more reliable marker of the B-lineage (24).

This methodology also provides a sensitive means of lymphoma detection (25). The technique detects about one tumor cell in the presence of 100 normal cells. Since only 10-20 micrograms of DNA are necessary for such an analysis, this procedure can be performed on about one million lymphocytes. This sample size can easily be obtained from fine needle aspiration biopsies (25). The technique is also useful in assigning lineage to histologically confusing tumors.

Cytogenetic analysis has also contributed important fundamental and practical information in classification of non-Hodgkin's lymphomas. The most important nonrandom cytogenetic patterns observed in these tumors are summarized in **Table 5.**

CONSISTENT CHROMOSOME ABNORMALITIES ASSOCIATED WITH NON-HODGKIN'S LYMPHOMA[a]

Working Formulation Diagnosis	Chromosome Abnormality		
	Trisomy	Translocation	Deletion
Low Grade			
Malignant lymphoma, Small lymphocytic, consistent with CLL	12		
Malignant lymphoma, follicular Predominantly small cleaved cell		t(14;18)	6q
Malignant lymphoma, follicular Mixed, small cleaved and large cell	8	t(14;18)	2q
Intermediate Grade			
Malignant lymphoma, follicular Predominantly large cell	7	t(14;18)	
Malignant lymphoma, diffuse Small cleaved cell			8p, 20q
Malignant lymphoma, diffuse Mixed, small and large cell	3		
Malignant lymphoma, diffuse Large cell	7, 18		
High Grade			
Malignant lymphoma, Large cell, immunoblastic			
Malignant lymphoma, Lymphoblastic			
Malignant lymphoma, Small noncleaved cell		t(8;14)	

[a] From P. R. K. Koduru *et al.*[34a] (From Reference 26)

About 30% of small lymphocyte lymphomas/chronic lymphocytic leukemias contain the trisomy 12 abnormality. At least 50% of all follicular lymphomas demonstrate the t(14;18) translocation and this abnormality is detectable by molecular analysis in up to 90% of such tumors (C. Croce, pers. communication). Translocations involving the immunoglobulin and c-myc loci characterize high grade lymphomas such as small non-cleaved and immunoblastic subtypes. A number of other translocations involving the immunoglobulin heavy chain locus provide important clues to the site of genes that regulate lymphocyte growth ie lymphocyte oncogenes. One of these loci is found on chromosome 11 (14). We are analyzing another such locus found on chromosome 2 band p13 which was involved in two unique cases of adolesent chronic lymphocytic leukemia seen by Dr. George Buchanan at Children's Medical Center (27,28). Recombination involving immunoglobulin gene loci is not suprising in view of the normal genetic rearrangement events which take place during B-cell development. There is still debate, however, whether these loci are selected by homologous recombination, growth advantage or both. If these tumors represent selection of rare genetic rearrangements which confer a growth advantage over normal cells, then analysis of these translocations will become a powerful means of isolation and characterization of lymphocyte growth-regulatory genes.

In summary, immunophenotypic genetic and cytogenetic methods of analysis are at present auxiliary means of classification of non-Hodgkins lymphoma's. Their importance will increase dramatically in the future as means of improving detection, understanding pathogenesis and devising novel treatment schemes aimed at underlying molecular abnormalities.

Improvements In Detection and Staging

One may legitimately ask whether refinements in staging procedures are necessary in evaluating patients with non-Hodgkins lymphoma's. Only 5-10% of patients with follicular lymphoma and 30% with diffuse large cell lymphoma wind up with early stage (I or II) disease after standard procedures including lymphangiography or CT scanning, bone marrow biopsy and liver biopsy. Careful clinical staging is indicated as a baseline to follow treatment efficacy. Routine procedures are summarized in **Table 6.**

Staging Hodgkin's Disease and Non-Hodgkin's Lymphomas: Required Evaluation Procedures

1. Adequate surgical biopsy, reviewed by an experienced hematologist
2. A detailed history recording duration and the presence or absence of fever, unexplained sweating and its severity, unexplained pruritus, and unexplained weight loss
3. A careful and detailed physical examination; special attention to all node-bearing areas, including Waldeyer's ring, and determination of size of liver and spleen
4. Necessary laboratory procedures
   a. Complete blood count, including an erythrocytic sedimentation rate
   b. Serum alkaline phosphatase
   c. Evaluation of renal function
   d. Evaluation of liver function
5. Radiologic studies include
   a. Chest roentgenogram (PA and lateral)
   b. Intravenous pyelogram
   c. Bilateral lower extremity lymphogram
   d. Views of skeletal system to include thoracic and lumbar vertebrae, the pelvis, proximal extremities, and any areas of bone tenderness

Staging Hodgkin's Disease and Non-Hodgkin's Lymphomas: Procedures Required Under Certain Circumstances

1. Whole-chest tomography if any abnormality is noted or suspected on the routine chest roentgenogram
2. Abdominal CT scan, ultrasonogram, inferior cavography, or pyelogram to supplement lymphographic findings. CT scans are particularly useful and the only reliable technique for identifying mesenteric nodes in non-Hodgkin's lymphomas.
3. Bone marrow *biopsy* (needle or open) in the presence of
   a. An elevated alkaline phosphatase
   b. Unexplained anemia or other blood count depression
   c. Other evidence of bone disease (scan or x-ray)
   d. Generalized disease of stage III or greater

Useful Ancillary Procedures Not Required for Staging

1. Skeletal scintigrams*
2. Hepatic and spleen scintigrams*
3. Serum chemistries to include serum calcium and uric acid for overall management of patient
4. Estimates of the patient's delayed hypersensitivity of the tuberculin type
5. Gallium whole-body scans*

* Cannot be used as evidence of Hodgkin's disease without biopsy confirmation

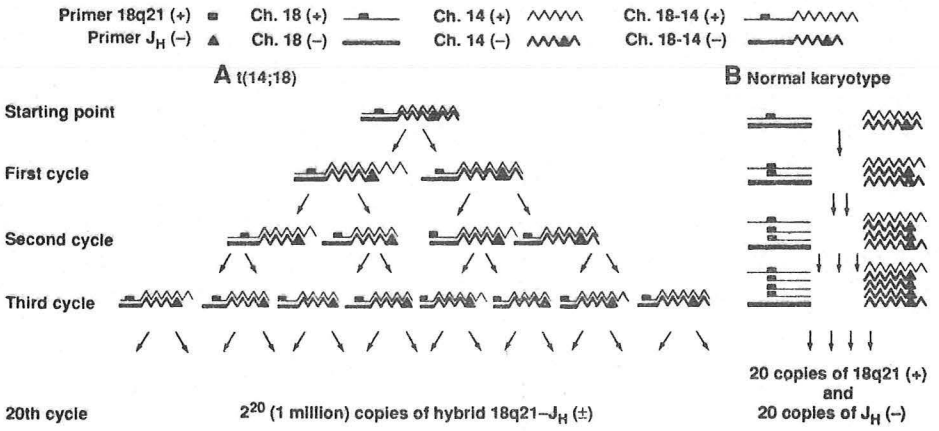
(From Reference 16a)

Radiographic imaging procedures for the staging of lymphoma have been refined. CT scanning of the chest may play a constructive role. In one study, about one quarter of the patients with negative chest x-rays had unequivocal disease demonstrated by CT scanning (29). CT scanning of the chest changed management in about one quarter of untreated patients, especially those with Stage I or Stage II disease. Although bone lesions are common in patients with diffuse large cell lymphomas, they are rarely the only sign of extranodal disease and therefore routine bone scanning is not a particularly useful staging procedure (30). However, the bone scan is quite positive in localizing skeletal disease. MRI scans have demonstrated potential for localizing areas of marrow involvement for biopsy (31). Since marrow involvement is often focal in non-Hodgkin's lymphoma, the value of MRI scanning for this purpose should be studied further. The problem of extent of gastric involvement and operability in diffuse large cell lymphoma occasionally is an important clinical issue, since a complete resection correlates with favorable long-term outcome (32). In a report from the Netherlands, Tio et.al. demonstrated that endoscopic ultrasonography was more accurate than CT scanning in defining the extent of gastric and adjacent lymph node involvement and therefore predicting resectability (33).

Genetic techniques are certain to make a contribution to staging in the future. As mentioned above, immunoglobulin gene rearrangement analysis on fine needle aspirate material can provide an important adjunct in diagnosis and staging. The cytologic diagnosis of lymphocytic lymphomas in such aspirates is notoriously difficult, since there are often no reliable cytologic features that distinguish normal from neoplastic lymphocytes. Hu and co-workers (25) have shown that clonal immunoglobulin gene rearrangements can easily be detected in as few as one million cells recovered from such an aspirate. When DNA is available from an open biopsy which demonstrates lymphoma in such a patient, the specific rearrangements in open and fine needle biopsies can be compared to establish the presence of tumor in the fine needle aspirate. If no open biopsy is available, the presence of clonal rearrangements is strong presumptive but not conclusive evidence of B-cell lymphoma. Obviously, such information would have to be integrated into the total clinical and pathological secture for decision making. This technique may be valuable for extensive staging of masses not easily accessible to open biopsy.

A sensitive new genetic technique has been developed for the detection of DNA sequences that are unique to tumor cells. Such sequences would be a particularly valuable markers for malignancy if they were present in the majority of tumors of a given type. Such a sequence is present in follicular lymphoma and is formed

This translocation creates a new sequence which is not found in any normal cell. The technique is known as polymerase chain reaction and it can detect as few as one abnormal cell in a million (34). DNA is extracted from test cells and hybridized to two oligonucleotide primers. These primers anneal to sequences on chromosomes 18 and 14 that span the break point. DNA polymerase is then added which exponentially amplifies the sequence between the two primers; that is, the sequence which is unique to the tumor (**Figure 5**).



Schematic illustration of the mechanism by which PCR preferentially amplifies the hybrid 18q21–J_H DNA sequences, but not the normal DNA sequences. (**A**) In case of the t(14;18), the hybrid 18q21–J_H(+) and 18q21–J_H(−) DNA sequences were synthesized from primer 18q21(+) and primer J_H(−), respectively. The primers are also complementary to the newly synthesized hybrid 18q21–J_H(±) DNA sequences, which, in turn, become templates for the primers. Therefore, exponential amplification of the hybrid 18q21–J_H(±) DNA sequences are generated, that is, $Y = (1 + E)^N$, where Y is the extent of yield, E is the mean efficiency per PCR cycle, and N is the number of PCR cycles carried out. If E = 100% and N = 20, the final yield is 2^{20} copies of hybrid 18q21–J_H(±) DNA sequences. (**B**) In case of a normal karyotype, the newly synthesized 18q21(+) and J_H(−) DNA sequences cannot be templates for the primers. Therefore, the final yield is calculated as the following formula: $y = 2n \times e$, where y is the extent of yield, n is the number of PCR cycles, and e is the mean efficiency per cycle.

(From reference 34)

Recent refinements of this technique exploit the properties of a heat-stable DNA polymerase which allows the reaction to be carried out at 65° (35). This is important because the priming is highly specific at this temperature, and because the thermostable enzyme allows the reaction to be carried out in a single incubation period rather than multiple cycles of polymerization. In the end, the amplified sequences are analyzed by Southern blotting and hybridization to probes derived from both chromosomes 18 and 14. Since the chromosomal translocation is a clonal marker, identification of a specific amplified fragment confirms the presence of tumor DNA in the sample. The sensitivity of detection is far above that of any other technique so far devised. For the first time, this procedure should allow us to study true minimal residual disease in the follicular lymphomas. As I will discuss shortly, ongoing clinical trials are designed to ask whether "third generation" treatment regimens can eradicate follicular lymphoma, which has traditionally been

considered incurable with standard chemotherapy. A sensitive assay for minimal residual disease would help understand the course of treatment failure and assist in the design of new trials.

Each of these newer technologies must be tested in prospective clinical trials to establish their role as a guide to management. Ultimately, each new technology must be tested in context with established techniques to ask whether the new procedure can refine treatment to enhance survival. The challenge for the immediate future, then, is to integrate these detection and staging procedures into prospective clinical trials. Initially, we must correlate the results of the procedures with clinical events. If these correlations are strong, further trials must be planned to test the validity of altering management based on results of these tests.

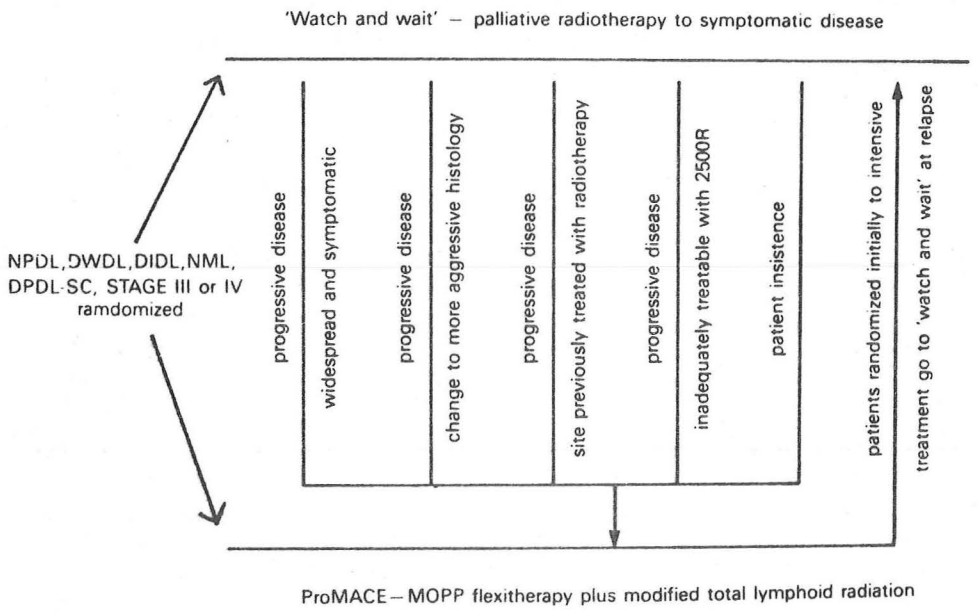
Current Trends in Therapy

Should indolent lymphomas be treated conservatively or aggressively?

The argument for conservative therapy of low-grade lymphomas gained strength from two observations. First, although a majority of patients achieve complete remission with standard chemotherapy such as CVP, almost all of these patients will relapse within four years (36). In fact, no known chemotherapy regimen has been shown reproducibly to cure these lymphomas. Second, the median survival of these indolent lymphomas is around seven years. These observations led to a policy of "watchful waiting" in the management of these patients, a approach championed primarily by the Stanford school (37). On the other hand, a number of recent observations challenge this "do no harm" approach. First, certain subsets of patients may have a significantly shorter survival and therefore may be appropriate candidates for aggressive therapy. These subsets include patients with neoplastic plasma cells as a component of their follicular lymphomas (38), patients with advanced stage disease (39) and patients with B symptoms, anemia, abnormal liver function tests or hepatosplenomegaly (40). Patients in these subsets had a median survival of less than five years with conservative therapy. Second, some patients with follicular lymphoma are clearly curable. In particular, the small subset of patients with stage I or II disease may do well with extended field radiation. In one trial, the actuarial ten year relapse free survival in patients so treated was 83% (41). At the M.D. Anderson Hospital, about half of stage I and II follicular lymphoma patients were judged curable and the 5 year relapse-free survival rates were better in patients receiving chemotherapy with or without radiation (64%) than in those treated with radiation therapy alone (37%) (42). Similarly, the Roswell Park

group obtained an 83% disease-free survival at 10 years in stage I and II patients treated only with radiation therapy (43). Thus, clearly some patients even with stage II follicular lymphoma are curable. The major question remains whether the vast majority of patients who have advanced stage disease are curable with any chemotherapeutic approach.

In attempt to answer this question, the NCI group is performing a prospective randomized trial comparing conservative treatment, consisting of a "watch and wait" policy of palliative radiotherapy only for symptomatic disease, with aggressive ProMACE-MOPP chemotherapy followed by 2,200 to 2,500 rads of total lymphoid radiation for patients who achieve complete clinical remission (44,45) (**Figure 6**).



Schema for National Cancer Institute "Good Prognosis" lymphoma study.
(From Reference 44)

Patients on conservative therapy whose disease progresses are crossed over to chemotherapy, and patients who relapse on chemotherapy are then treated conservatively. This trial has been active for about seven years, and given the prolonged history of these lymphomas no final conclusions can yet be drawn. However, some interesting interim observations have been published. At present there are no significant survival differences between patients randomized to either arms of management. Patients randomized to aggressive therapy achieved a 76% (25/33) complete remission rate, with 84% of complete responders remaining in continuous complete remission for a median in excess of four years (45). This exceeds the best previously reported results, specifically median duration of remission about two years. Patients who initially received conservative management responded less well when crossed over to aggressive chemotherapy; only 44% (7/16) of these patients

achieved complete remission. Two of these 7 patients relapsed within 1 year. Patients who received delayed aggressive management in a University of Chicago study also achieved a lower complete remission rate (25%) than did those receiving initial aggressive therapy (71%) (46). In the NCI study, the median time to crossover to aggressive management was 26 months; such crossover was necessary in 38% of the patients. Six patients who received no cytotoxic therapy suffered histologic conversion to large cell lymphoma, confirming that such progression is part of the natural history of follicular lymphoma. The toxicity of this combined modality program included pneumocystis pneumonia, myelodysplasia and second neoplasms each in about 5% of patients. The tentative results suggest that aggressive initial management alters the timing of relapse. It is still too early to judge whether this approach will provide long term control of disease. The preliminary data also suggest that a delay in application in aggressive therapy reduces its efficacy. This outcome is predicted by the Goldie-Coldman hypothesis, which assumes that tumors develop multiple drug-resistant subclones in a random stepwise fashion over time (47).

These prospective trials are important not only for testing efficacy of modern dose intensive treatment regimen but also for advancing our understanding of the natural history of indolent lymphomas. At the very least, these studies will lead to sharpened hypotheses to explain the underlying reasons for treatment failure. An important aspect of future studies will be an analysis of drug resistance in the subclones which survive initial cytotoxic therapy. The molecular basis of drug resistance must be understood.

Academic and private practice oncologists must cooperate in enrolling patients with indolent lymphomas into clinical trials. This recruitment has become more difficult in recent years as the concept of conservative therapy has gained widespread acceptance. Clearly, for at least certain subsets of patients with "indolent" lymphomas, conservative therapy may not be in their best interest.

<u>What is the optimal management of aggressive (large cell diffuse, immunoblastic) lymphoma?</u>

A consensus is gathering regarding the management of <u>early stage</u> (I and II) diffuse large cell lymphoma. In stage "E" disease, as much tumor as possible should be resected prior to cytotoxic therapy. Localized radiation therapy is inadequate to cure the majority of these patients (48). The addition of short course, dose intensive chemotherapy to radiation therapy has been associated with prolonged disease-free survival in the vast majority of patients (49). Clearly, after resection, multiagent chemotherapy should be the next step in management of these patients. The decision regarding follow-up involved field

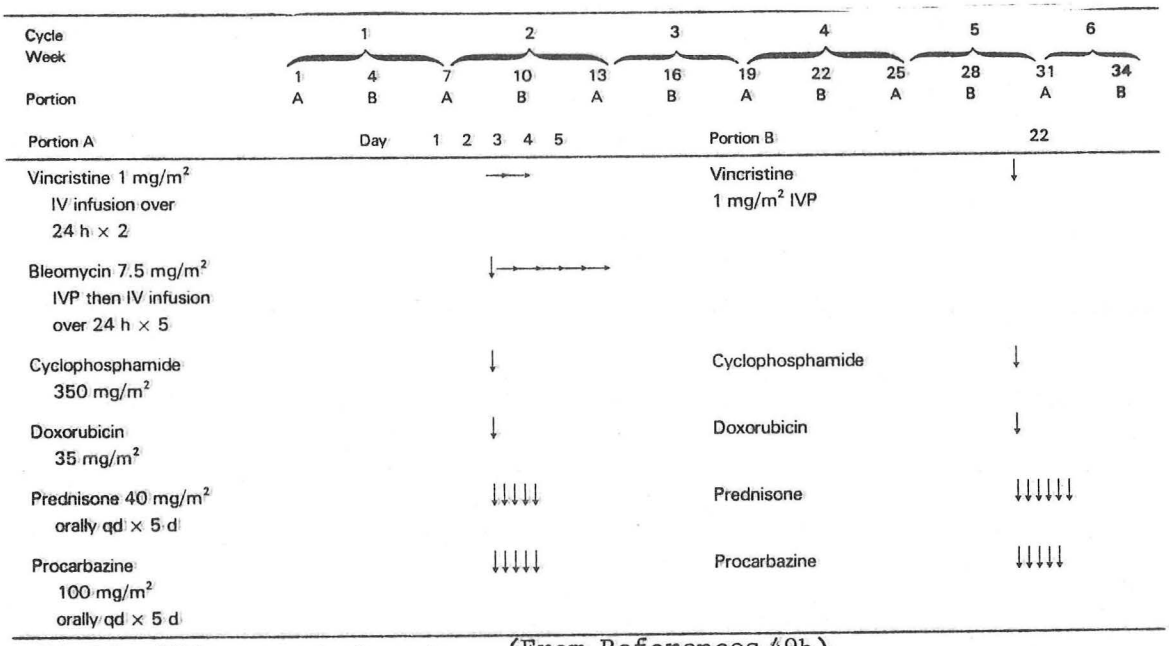
radiation therapy turns upon the volume of initial and residual disease.

The biology of the advanced stage diffuse large cell lymphoma includes relentless progression to the death of the patient within one to two years, unless the disease is eradicated. Over the last 15 years several "generations" of multiagent chemotherapy programs have evolved with the aim of increasing complete remission rates and long term survival. At the present time, three such "third generation" programs compete for supremacy in this regard (**Table 7**).

ProMACE-CytaBOM	Day 1	Day 8	Day 14	Days 15–21
				No therapy
Cyclophosphamide 650 mg/M^2 IV	x			
Doxorubicin 25 mg/M^2 IV	x			
Etoposide 120 mg/M^2 IV	x			
Cytarabine 300 mg/M^2 IV		x		
Bleomycin 5 mg/M^2 IV		x		
Vincristine 1.4 mg/M^2 IV		x		
Methotrexate 120 mg/M^2 IV		x with leucovorin rescue		
Prednisone 60 mg/M^2 PO	x----------x			
Cotrimoxazole 2 PO BID throughout 6 cycles of therapy				

MACOP-B	Week	1	2	3	4	5	6	7	8	9	10	11	12
Cyclophosphamide 350		x		x		x		x		x		x	
Doxorubicin 50 mg/M^2 IV		x		x		x		x		x		x	
Vincristine 1.4 mg/M^2 IV			x		x		x		x		x		x
*Methotrexate 400 mg/M^2 IV			x				x				x		
Bleomycin 10 mg/M^2 IV					x				x				x
Prednisone 75 mg/M^2 PO QD		x----------											-taper
Cotrimoxazole 2 PO BID		x----------											----x

* With leucovorin rescue. (From References 49a)



Abbreviations: IVP, intravenous push; qd, every day. (From References 49b)

(TABLE 7)

	COP-BLAM (I)	COP-BLAM III	ProMACE CytaBOM	MACOP-B	High-dose Doxorubicin†
Percent CR	73	84	80	84	91
Survival plateau (%) (patients alive, well, off treatment)	55	65	63	63	64
Potential cure (%) (excluding inadvertent deaths)	60	76	74	70	77
Treatment time (mo)	6	8	4-6	3	4
Percent of patients >60 yr	50	51	NA	29	NA
Percent of patients >70 yr	17	24	NA	1	0

Abbreviations: NA, not available.

*Data as best derived from multiple sources.

†Includes nonbulky stage II patients.

(From References 49b)

The primary innovation of the third generation programs is the delivery of chemotherapy in a more dose-intense fashion. The hypothesis here is that exposure to more chemotherapy (drug concentration x time) over a shorter overall treatment period will be associated with lower risks of drug resistance and treatment failure.

At the present time, published information is in disagreement regarding the efficacy of the MACOP-B regimen. In the original Vancouver series of patients, complete remission rates of 83% were obtained and 65% of all patients remain in initial complete clinical remission with a median follow-up of 48 months (50). On the other hand, the Southwest Oncology Group achieved complete remission in only 50% of patients (51). SWOG observed more severe toxicity than did the Vancouver group, including severe granulocytopenia (48%), mucositis (23%), and thrombocytopenia (9%). The Southwest Oncology Group is attempting to answer the important question whether MACOP-B is superior to the first generation regimen CHOP, the second generation program m-BACOD and to the third generation regimen ProMACE-CytaBOM (51). Randomized comparisons of dose-intensive regimens with first and second generation programs are important because the toxicity of the third generation combinations is formidable. Our own experience as well as that of others (52,53) clearly indicates that patients over age 60 tolorate MACOP-B less well than younger patients. It is quite probable that variations in prognostic factors such as age, performance status, volume of disease and number of sites involved account for differences in results in different trials (**Table 8**).

TABLE 8 FACTORS PREDICTING TREATMENT FAILURE IN DIFFUSE LARGE CELL LYMPHOMA

Factor	Rx	Reference
Poor performance Status, bulky disease >10cm, >2 extranodal sites of disease.	M,m-BACOD	54
Age > 56, B symptoms, LDH > 225 IU/L, > 3 extranodal sites of disease.	CHOP-Bleo	55
Age > 55 poor performance Status, B Symptoms.	CHOP	56
Abdominal mass > 10cm, B symptoms extra nodal involvement, LDH > 600 IU/L	CHOP	57

Several of these factors appear to be independent predictors of outcome and have therefore been proposed to form the basis of an improved staging system for aggressive lymphomas (58) (**Table 9**).

Proposed New Staging Schema for Aggressive non-Hodgkin's Lymphomas

Stage I	Involvement of a single lymph node region or a single extralymphatic organ or site without node involvement (IE)
Stage II	Involvement of two or more lymph node regions or localized extralymphatic organ or site (IIE) with none of the following poor prognostic signs: poor performance status (ECOG 2–4), mass larger than 10 cm, 3 or more extranodal sites, marrow involvement, LDH greater than 500
Stage III	Presence of one or more of the poor prognostic signs

A = asymptomatic; B = fever, sweats, unexplained weight loss greater than 10 percent of body weight.

(From References 49a)

In summary, the gradual improvement in complete remission rates over the past 15 years suggests that dose-intensive therapy is an appropriate concept in the management of difusse large cell lymphoma, but these programs must be tested against less intensive treatments in prospective randomized trials.

Immunologic and Biologic Approaches to Therapy

Autologous Bone Marrow Transplantation For Relapsed Non-Hodgkins Lymphoma.

Chemotherapeutic salvage of patients with aggressive lymphomas in relapse has been unsatisfactory (59). Therefore, a number of groups have resorted to high dose chemotherapy with or without total body radiation followed by infusion of cryopreserved autologous bone marrow for the management of such patients (60,61). The results of these trials suggest that this approach is useful when disease burden is moderate and the tumor retains at least partial sensitivity to standard chemotherapy. A major potential limiting problem is the presence of tumor cells in the bone marrow. The group at the Dana Farber Cancer Center has approached this problem by purging marrow with anti-CD20 antibody and complement, which kills all B-cells in the marrow (60). Since this marker is found on almost all B-cell lymphomas, such treatment should rid the bone marrow of most of the tumor cells. Nadler and co-workers have treated 125 patients with high-dose Cytoxan and total body radiation followed by infusion of autologous marrow "purged" in this fashion. Hematologic reconstitution was complete within a few weeks. However, immunologic reconstitution, including recovery of circulating T and B-cells and immunoglobulin levels, required more than a year in many patients (62). The requirement for bone marrow purging is by no means clear since randomized trials to compare treated and untreated bone marrow have not been done. However, patients with clear-cut marrow involvement at the time of harvesting have in general not relapsed in the bone marrow following reconstitution. The major problem is recurrence of lymphoma in other sites. Nonetheless, 50% of these patients are alive and free of disease at a median follow-up of five years, and it is estimated that the procedure will cure about 25% of the total group (63; L. Nadler, pers. communication). There were 7 deaths in the early post-transplantation period. The procedure is certainly worthy of a wider trial for patients with low-burden relapses which retain at least partial sensitivity to available drugs. Unfortunately, patients who present in relapse with bulky disease that is refractory to standard chemotherapy cannot be salvaged with any known treatment program. Again, a major current limitation is lack of a useful understanding of mechanisms of drug resistance.

Anti-idiotypic therapy of non-Hodgkin's lymphomas

We have known for years that indolent lymphomas are difficult if not impossible to eradicate with conventional chemotherapy. One possible reason is the kinetic inactivity of a large fraction of these cells. Persistence of indolent disease is thought to present an ongoing risk of transformation to high grade lymphoma. (64). One approach to overcoming these problems is an immunologic attack upon the lymphoma cells aimed at the only

known tumor-specific antigen, namely the idiotype associated with the membrane immunoglobulin of these B-cell tumors. Using monoclonal antibody technology, Ron Levy and coworkers at Stanford have pioneered this approach in patients with follicular and other indolent B-cell lymphomas. His index patient, a man with drug-resistent follicular lymphoma, was treated in 1981 with anti-idiotypic antibody (65). This patient remains in continuous complete remission seven years later. Levy has reported on 9 additional patients, 5 of whom exhibited a partial response and 4 no response. In a series of careful studies, Levy's team have defined some of the variables which correlate with success and failure (66). A major problem is mutation in the idiotypic regions of the tumor cells, resulting in loss of the idiotope seen by the monoclonal antibody (67). In retrospect, Levy could not have selected a more difficult B-cell tumor to control with anti-idiotypic antibody. It is now known that in normal germinal center B-cells, immunoglobulin variable region genes extensively mutate apparently to provide a basis for selection of clones with high antibody affinity. Thus it is not surprising that the neoplastic counterpart, the follicular lymphoma cell, would also rapidly mutate in this region with consequent loss of idiotopes. This process may be accelerated under the selective pressure of anti-idiotypic antibody. In short, variants can quickly emerge which no longer bind the theraputic antibody. Using hybridoma technology and variable region sequence analysis, Levy and coworkers have correlated loss of idiotopes with specific variable region gene mutations in these variants (67). However, almost all variants continue to express membrane immunoglobulin, leading to the speculation that the membrane immunoglobulin provides some selective advantage for the tumor cell population. The nature of this advantage is not clear; possibilities include internal antigenic or anti-idiotypic network stimulation of growth. Analysis of the entire group of patients has shown that anti-tumor response correlates with neither the isotype of the anti-idiotypic antibody used nor its affinity. Antibodies directed against heavy-light chain combinatorial idiotope determinants were more likely to yield a therapeutic response. In one patient, the anti-idiotypic antibody had a direct antiproliferative effect on the tumor cells. However, in the majority of patients, therapeutic outcome correlated with the number of normal CD4 bearing (helper-inducer) host T-lymphocytes in the lymphomatous nodes. This correlation suggests that a host anti-tumor response, perhaps triggered by anti-idiotypic antibodies but also involving normal T lymphocytes is essential for a successful outcome. A prediction of this hypothesis would be that combined anti-idiotypic and adoptive T-cell immunotherapy (68) would enhance the number of complete responses. Levy is now isolating these T-cells directly from involved lymph nodes to study their interaction with the neoplastic B-cells.

Immunotoxin Therapy

Another approach to killing the slowly cycling or G_0 neoplastic B-cell is immunotoxin therapy. Drs. Ellen Vitetta and Jonathan Uhr of our Center have been interested in this approach in mouse lymphoma models and more recently in man (69). A potent biological toxin, for example the A chain of ricin, is coupled to an antibody which binds specifically to B-cells. Currently Drs. Vitetta and Uhr have selected CD22 as this target because some of its epitopes are expressed only on B-cells and not on any other normal cell. The advantage of selecting a differentiation marker such as CD22 is that unlike surface idiotype, new antibodies do not need to be generated for each patient treated. This is significant, since Levy's anti-idiotypic antibodies take about a year to ready for clinical use. Of course the disadvantage of lineage-specific antibodies is the destruction of normal B-cells. However, B-cells are able to regenerate from precursors normally present in bone marrow. The chemistry of toxin-antibody linkage has been progressively refined. A major roadblock has been nonspecific sequestration of conjugate in the liver. This problem has been overcome by the use of recombinant toxin which is free of mannose side chains. Several monoclonal antibodies have had to be screened since only a few make highly effective immunotoxins for reasons of variation in isotype, epitope and affinity. At the present time the toxicology of the conjugates IS being studied in primates.

It is already clear from animal studies that this approach will be successful only when used in a setting of minimal residual disease. This, however, is just the setting which presents the limiting problem for chemotherapy in the follicular lymphomas: complete remissions are easy to obtain but impossible to maintain with cytotoxic chemotherapy. The results of these trials should indicate whether the immunotoxin approach can contribute to the solution of this problem.

A major theoretical limitation of the approach is immune selection of variants which lack the CD22 marker. Ultimately, it may be necessary to generate a panel of immunotoxins which recognize a broader range of B-lineage differentiation markers. In this way, the emergence of variants may be preventable.

Hopes for the Future

At the present time, several promising leads beckon the clinical investigator. As I have mentioned, combinations of conventional cytotoxic drugs with immunologic therapy consisting of antibodies and effector cells hold significant promise for control of minimal residual disease. Additional advances in understanding the growth and development in B lymphocytes may also lead to novel therapies. Specifically, no less than 8 polypeptide growth factors have now been isolated and cloned which influence the growth and/or differentiation of these cells (**Table 10**).

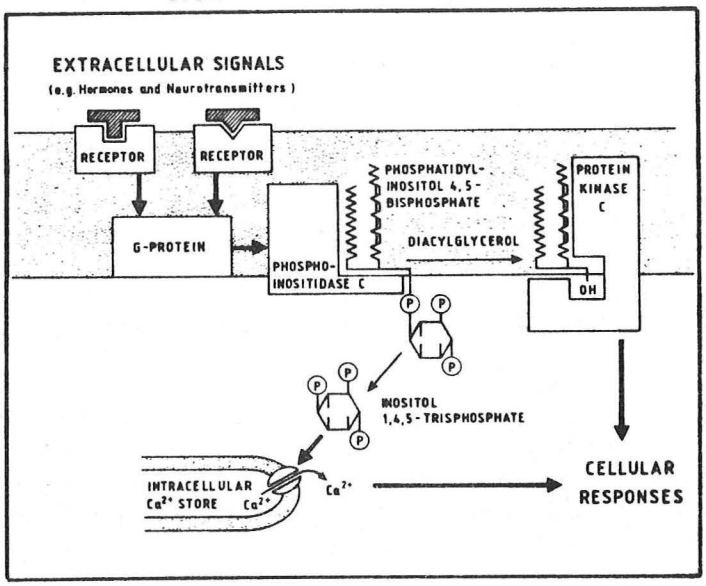
TABLE 10 POLYPEPTIDES ACTING ON B LINEAGE CELLS

Factor	Cloned from	MW (kDa)	Sources	Actions on B-Cells	Refs
IL 1	H,M	17.5	Macrophage, many other cells	Growth/differentiation factor for B-cells. Synergy with Il 4,5 in growth/ differentiation.	70
IL 2	H,M	15.5	Activated T cells (T_{H1} subset)	Proliferation and differentiation.	71
IL 3	H,G,M	25(140 aa)	Activated T cells	Supports growth of B cell (or multipotent) precursors.	72
IL 4	H,M	20	Activated T cells (T_{H2} subset)	Costimulates mouse (may inhibit human*) B cell prolif./differentiation Stimulates production of IgG1 and IgE, blocks IgG2a secretion (mouse).	73
IL 5	H,M	45 (23x2)	Activated T cells ($T_{H2}>T_{H1}$)	Promotes differentiation of activated B cells to Ig secreting stage (esp IgA). Induces proliferation of cells at certain maturation stages.	74
IL 6	H,M	21	Activated T Cells other cells	Promotes differentiation of activated B-cells to Ig secreting cells. Autocrine growth factor for myeloma cells.	75
IFN ɣ	H,M	20,25(146 aa)	Activated T cells	Promotes differentiation of activated B cells to Ig secretion, especially IgG2a isotype. Blocks IgG1 and IgE secretion. Increases class II MHC expression.	71,76
Lympho-poietin 1	M	25	Marrow stromal cell line	Supports growth of B-cell precursors.	77

IL, Interleukin; IFN, interferon; H, human; M, mouse; G, gibbon; aa, amino acids; MHC, major histocompatibility complex. T_{H1} and T_{H2} are helper T cell subsets that have been defined in the mouse.
*Peter Lipsky, personal communication.

These interleukins affect proliferation and maturation of B-cells at different developmental stages. They interact with a host of cells in addition to B lymphocytes, and their true in vivo functions are unknown. Most of our current information comes from studies in the mouse. However, recombinant human factors are available and we can expect a rapid accumulation of human data in the near future. The relevance of some of these factors to tumor cell growth is already emerging. For example, IL-6 is a potent growth factor for myeloma cells in vitro (75). The majority of myelomas tested express both IL 6 and its receptor, and cell growth is inhibited by the addition of anti-IL 6 antibody. Certain human B-cell lymphomas also release growth factors which self-stimulate proliferation (78,79). In the future, antagonists of these growth factors may become important anti-lymphoma drugs. Such antagonists may be particularly useful when cycled appropriately with chemotherapy. The growth factors themselves could be used to recruit indolent lymphomas into growth cycle, thus rendering them more sensitive to cycle active chemotherapy.

A number of investigators have shown that at least some of the biochemical pathways which transduce external growth signals throughout B lymphocytes are basically similar to the mechanisms well described in other types of cells (**Figure 7**) (80).



Inositol Lipids in Cellular Signaling

(From Reference 80a)

Specifically, the hydrolysis of inositol phospholipids is well documented as a key step in many signal transduction systems. These inositol phosphates and diacylglycerol are thought to release stored intracellular calcium and to activate protein kinase C, respectively. The membrane bound phosphoinositidase C catalyzes this hydrolysis. Phosphoinositidase C is modulated by guanine nucleotide-binding (G) proteins in an interaction which is quite analogous to the regulation of adenyl cyclase (81). The activation of these pathways can be followed in B-cells after delivery of external signals acting through several cell surface receptors such as membrane immunoglobulin and Fc receptors (82). Further advances in understanding these intricate pathways of growth signal transduction will certainly provide useful clues as to where to look for abnormalities in growth control in B-cell lymphomas. Ultimately, this sort of understanding should lead to a whole new class of antiproliferative drugs which act at various steps along these pathways. Although we are currently ignorant of the intermediate steps, growth signal transduction must eventually activate gene transcription which is known to precede cell growth and division. The nuclear proteins c-myc and c-fos are induced following growth activation of a variety of cells including lymphocytes (83,84). Since c-myc is also a powerful oncogene for B-cells in a variety of species, including man, further studies of the regulation and functions of these proteins should help unravel this end of the growth signal transduction pathways.

Summary

The major therapeutic questions addressed in this Grand Rounds should be answered within the next 5 years as the results of well planned clinical trial become available. We should learn whether indolent lymphomas can be cured with aggressive third generation chemotherapy programs. If not, we should focus upon combinations of chemotherapy for cytoreduction and immunologic control for management of minimal residual disease. In the case of diffuse large cell lymphomas, we should know whether the application of dose-intense regimens cures a higher fraction of cured patients.

Perhaps in more than any other lineage, rapid advances in understanding of regulation of growth and development of normal cells sets the stage for exciting new developments in the management of malignancies. With hybridoma technology, we can capture the tumor and its subclones for genetic analysis. The same hybridomas can provide a ready source of the major unique markers for these tumors, namely clonotypic immunoglobulin molecules which may serve as therapeutic targets. Recombinant DNA technology has led to a detailed characterization of unique molecular abnormalities such as chromosomal translocations. These sequences will provide additional specific clonal markers for monitoring lymphomas. Advances in understanding of signal transduction pathways in B-cells and regulatory interactions with other cells will provide us with many important leads for future investigation. The major challenge for the clinical scientist will be to recruit sufficient patients into trials to test these ideas.

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