Distinct Cytogenetic and Clinicopathologic Features in Acute Myeloid Leukemia After Occupational Exposure to Pesticides and Organic Solvents

Franca Fagioli, M.D., Antonio Cuneo, M.D., Nadia Piva, M.D.,

Maria Gretel Carli, Ph.D., Rita Previati, M.D., Massimo Balboni, M.D.,

Paolo Tomasi, M.D., Daniele Cariani, M.D., Gianluigi Scapoli, M.D.,

and Gianluigi Castoldi, M.D.

Background. To study the correlation of environmental exposure to potentially mutagenic agents and the clinicopathologic picture in acute myeloid leukemia (AML), clinical features, morphologic characteristics, immunophenotype, and cytogenetics were studied in 59 patients with newly diagnosed AML.

Methods. Based on interviews on occupational hazards and hobbies showing prolonged contact with pesticides (18 patients) and organic solvents (7 patients), 25 patients were categorized as "exposed." Thirty-four patients were categorized as "unexposed," based on anamnestic findings.

Results. Light microscopic studies showed myelodysplasia involving multiple cell lineages in all assessable patients with professional exposure to pesticides and organic solvents, whereas morphologic aberrations of the non–blast cell population were confined to a minority of cells in unexposed patients. These findings were confirmed by electron microscopic studies in 31 patients. Immunologic analysis showed the presence of a minor megakaryoblastic component in six exposed patients and showed positive findings for the CD34 stem cell marker in 85% of exposed patients, a figure significantly higher as compared with that for unexposed subjects. Cytogenetic studies confirmed the frequent occurrence of 5q and/or 7q aberrations in patients occupationally exposed (10 of 25 cases). Other recurring chromosome aberrations in the exposed group were 17p−, trisomy 11q, and translocation of 16q, 6p, 7p, and 11p, whereas the classic AML-specific translocations (i.e., t[15;17]; t[8;21]) were detected only in unexposed subjects. Conventional chemotherapy achieved complete remission in 1 of 19 exposed patients, as opposed to 14 of 29 unexposed patients, with a median survival of 2 months in the former group and 8 months in the latter.

Conclusions. Taken together, these findings document that AML in patients professionally exposed to toxic substances may represent a distinct cytogenetic and clinicopathologic entity. The clinicobiologic characteristics in these exposed patients are similar to the features of AML arising in patients with prior chemotherapy for another tumor, thus suggesting that similar transformation pathways may underlie leukemogenesis induced by cytotoxic drugs and by environmental exposure to some pesticides or organic solvents. *Cancer* 1992; 70:77–85.

Key words: AML, cytogenetics, pesticides, organic solvents.

Although the leukemogenic role of cytotoxic drugs and radiation exposure is well documented,[1] the importance of other myelotoxic agents present in the environment in the genesis of acute myeloid leukemia (AML) is ill-defined. Indeed, except for benzene,[2] evidence linking environmental exposure and AML is weak, and additional studies are needed before a definite role in the origin of AML can be assigned to other toxic compounds, such as organic solvents, and pesticides.[3]

Because patients with AML with prior cytotoxic treatment of another tumor almost invariably have a particular form of disease,[4] usually referred to as secondary leukemia (SL), it is reasonable to assume that if a causal link exists between environmental exposure and AML, "exposed" patients should exhibit distinct clinicobiologic features as compared with a control group including "unexposed" subjects. This hypothesis is supported by previous studies comparing cytogenetic findings in AML arising in patients occupationally ex-

posed and in patients classified as unexposed, based on anamnestic findings, which showed a distinctive pattern of chromosome aberrations in the former group, similar to that found in SL.[5,6]

To better define the role of environmental exposure in the genesis of AML, we studied cytologic, immunologic, and cytogenetic features in a series including 25 consecutive patients with AML with prolonged professional exposure to pesticides or organic solvents and 34 patients classified as unexposed, based on anamnestic findings. In our exposed patients, the identification of distinct cytogenetic and clinicopathologic features argues in favor of a possible etiologic role of some pesticides and organic solvents in the genesis of AML.

Patients and Methods

Patient Selection

Seventy consecutive patients with de novo AML, admitted to our institution since 1986, were classified with the French–American–British (FAB) system[7,8] on the basis of recommended cytologic investigations.[9] In each case, bone marrow (BM) and/or peripheral blood samples were submitted routinely for immunologic and cytogenetic analysis. In 31 patients, electron microscopic studies also were performed.

Fifty-nine patients with BM smears available for cytologic review and assessable immunologic and cytogenetic data form the basis of the current report.

Exposed and Unexposed Patients

On admission, all patients were interviewed about their occupational history and hobbies. Everyone was questioned on previous contact with organic solvents, pesticides, chemicals, metals, and petroleum products and categorized subsequently as exposed or unexposed. Exposed subjects were questioned carefully about the type and duration of exposure and were asked whether they used protection measures (e.g., masks, gloves, pressurized cabins) in the workplace. An "exposure index"[10] was calculated as follows: hours/day × days/year × years.

Cytologic Studies

Light microscopic studies. In addition to the use of recommended procedures for the classification of each case by the FAB system, BM smears were reviewed to assess the presence of associated myelodysplastic features. According to previously proposed criteria,[11] trilineage myelodysplasia (TMDS) was defined by the presence of more than 25% dysplastic erythroblasts and more than 50% abnormal granulocytes and megakaryocytes.

Ultrastructural studies. Electron microscopic studies were performed for 31 patients—10 exposed to pes-

ticides, 4 exposed to organic solvents, and 17 unexposed. For morphologic analysis and the detection of endogenous peroxidase activities, cells obtained from heparinized BM aspirate were processed in the fresh state (buffy coat) or after separation on a Ficoll–Isopaque gradient (1.077 g/ml) (Sigma Diagnostics, St. Louis, MO). Cell pellets were fixed in 1.25% glutaraldehyde in Gey's buffer (GIBCO, Grand Island, NY) for 10 minutes at room temperature. The material was incubated in the dark at 20°C for 60 minutes in a medium consisting of 20 mg of diaminobenzidine in 10 ml of 0.05 M tris–hydrochloric acid buffer containing 0.01 ml of 3% hydrogen peroxide (pH 7.6).[12] After fixation by osmium tetroxide, dehydration and embedding were performed according to standard procedures. The grids were counterstained with uranyl acetate and lead citrate. Serial ultrathin sections were examined by means of a Hitachi 800 electron microscope.

Immunophenotyping. BM cytospin preparations and/or peripheral blood smears were stained by an immunocytochemical method using alkaline phosphatase–anti-alkaline phosphatase complexes.[13] To minimize Fc nonspecific binding, the slides were incubated previously with rabbit serum (Dakopatts, Copenhagen, Denmark). Reactivity was tested to a panel of monoclonal antibodies purchased from various firms: HPCA-1 (CD34) (Becton-Dickinson, Mountain View, CA), My9 (CD33), My7 (CD13), My4 (CD14) (Coulter Inc., Hialeah, FL), GpIIb/IIIa (CD41) (Dakopatts, Copenhagen, Denmark), Leu-12 (CD19) (Becton-Dickinson), OKB-Calla (CD10), OKT16 (CD7), and OKT11 (CD2) (Ortho Diagnostic System Inc., Raritan, NJ). The cutoff point for positive results was 20%.

Cytogenetics. Chromosome analysis was performed at leukemia diagnosis in all patients. BM samples were cultured for 24 and 48 hours without mitogens. Synchronization with methotrexate and thymidine was performed. Metaphases were G-banded with Wright's stain.[14] Whenever possible, at least 10 karyotypes were studied in cytogenetically abnormal patients, and chromosome aberrations were described according to the International System for Human Cytogenetic Nomenclature (ISCN).[15] Patients showing no clonal aberration in at least 20 metaphases were classified as cytogenetically normal.

Results

Environmental Exposure

Twenty-five patients (18 farmers, 4 factory painters, 2 shoe factory workers, 1 beautician) were categorized as exposed, based on interviews showing unequivocal contact with pesticides, including carbamates and organophosphates[16,17] (18 cases), and organic solvents (7

cases). Direct exposure to pesticides for 8 hours a day occurred in these patients at least four times a month for 6 months a year and lasted for 10 years or more, whereas daily exposure to organic solvents lasted at least 4 years. The mean exposure index for patients exposed to pesticides and organic solvents was 20.000 hours and 38.000 hours, respectively. Sixteen patients were working actively when AML was diagnosed, whereas, in 9 patients, direct exposure had ceased 3–9 years before referral to our center. In 34 patients (13 homemakers, 12 white collar workers, 2 students, 1 teacher, 3 operators, and 3 farmers), referred to as unexposed, no evidence of exposure to myelotoxic agents was documented anamnestically.

Clinical Features

The relevant clinical data in patients exposed to pesticides and organic solvents and in unexposed patients are summarized in Table 1. Overall, patients categorized as exposed had a median age (64 years) similar to that of unexposed patients (66 years), whereas the male–female ratio was 3.2 in the former group and 0.9 in the latter. Except for median age, no important difference emerged when comparing clinical features in patients exposed to pesticides and those exposed to organic solvents (see Table 1).

As compared with the unexposed group, exposed patients had lower leukocyte counts and lower blast cell percentages in the BM. Erythroleukemia and megakaryoblastic leukemia were encountered more frequently among exposed patients, whereas other FAB subtypes of AML were distributed almost uniformly in both categories of patients.

The clinical outcome in our exposed patients can be summarized as follows: Of 19 patients treated with the AML protocol including an anthracycline drug and cytarabine, complete remission was obtained in 1 patient; 12 patients died of aplasia-related complications; 2 patients were totally unresponsive; and 4 patients attained only partial remission. Repeat courses of low-dose cytarabine were administered to five exposed patients, who were judged not suitable for aggressive chemotherapy because of cardiomyopathy, liver dysfunction, or advanced age and deep leukopenia. All five patients showed reduction of peripheral blast cell counts, resulting in disease control for 3–31 months (median, 6 months). One patient died before treatment was started.

Among unexposed subjects treated with standard myeloablative chemotherapy, complete remission was obtained in 14 of 29 cases. Seven patients attained partial response (defined by < 20% BM blasts and < 5% peripheral blood blasts). Six patients in this group died in the aplastic phase, and two were resistant to chemotherapy. Low-dose cytarabine was administered to five unexposed patients, who had reduction of peripheral blast cell counts for 1–10 months (median, 3 months). The overall median survival length was 2 months in the exposed group and 8 months in the unexposed group.

Cytologic Features and Immunophenotype

Morphologic findings. Among subjects exposed to pesticides and organic solvents, morphologic abnormalities affecting multiple cell lineages were apparent in 21 patients and were not assessable in the remaining 4 patients because of overwhelming blast infiltrate. Ab-

Table 1. FAB Classes, Clinical Features at Presentation, Response to Chemotherapy, and Survival in Patients With AML Exposed to Pesticides or Organic Solvents and Patients Not Exposed*

	Exposed		Not exposed
	Pesticides	*Solvents*	
FAB subtypes†	M1(1); M2(6); M4(6); M5(2); M6(1); M7(2)	M1(1); M2(1); M3(1); M4(1); M5(2); M6(1)	M1(3); M2(8); M3(2); M4(12); M5(8); M6(1)
Age (yr)	50–82 (68)	32–74 (55)	14–79 (66)
Hemoglobin value (g/dl)	5.0–10.5 (9)	5.4–9.5 (7.6)	5.6–13.7 (8.9)
Leukocyte count ($\times 10^9$/l)	2.1–83.9 (6.6)	1.3–11.0 (4.8)	1.0–249.0 (15.6)
Platelet count ($\times 10^9$/l)	20.0–488.0 (85)	18–243.0 (85)	26.0–240.0 (71)
% bls (PB)	4–80 (32)	3–70 (30)	2–95 (64)
% bls (BM)	30–90 (45)	34–85 (58)	35–90 (70)
CR‡	1/13	0/6	14/29
Survival (mo)	1–78+ (1,5)	1–8 (2)	1–36+ (8)

FAB: French–American–British; AML: acute myeloid leukemia; CR: complete response; bls: blasts; PB: peripheral blood; BM: bone marrow.
* Results are reported as variation ranges; median values are given in parentheses.
† Number of cases in each FAB category.
‡ Number of complete remisssions/number of patients treated with myeloablative chemotherapy.

normal cytologic features included the classic signs of dysmyelopoietic syndrome[18] (i.e., nuclear irregularities and defective hemoglobinization in the erythrocyte series, neutrophil hypogranulation, pseudo-Pelger forms, micromegakaryocytes, and large mononuclear megakaryocytes). According to the stringent criteria of Brito-Babapulle et al.,[11] 15 patients in this group could be classified as having AML with TMDS.

In the unexposed group, morphologic abnormalities of the non–blast cell population were confined mostly to the granulocytic lineage. A minority of morphologically abnormal megakaryocytes was detected in five patients, whereas dyserythropoiesis involving more than 25% erythroblasts was present in six patients. In this category, myelodysplasia affecting more than one cell lineage was seen in only seven patients, none of which fulfilled the cytologic criteria for the diagnosis of AML with TMDS.

The results of light microscopic analysis were confirmed by electron microscopic studies. Ultrastructural aberrations involving all hematopoietic lineages were present in 13 of 14 exposed patients (3 exposed to organic solvents and 10 to pesticides). One patient exposed to organic solvents showed abnormalities of erythroblasts and neutrophil granulocytes with only minimal aberrations of megakaryocytes. In agreement with previously reported findings,[19] the morphologic abnormalities observed most frequently in erythroid cells were nucleocytoplasmic asynchrony, vacuoles, and mitochondria with intracrestal accumulation of iron.[20] Neutrophil granulocytes exhibited decreased primary and secondary granule formation, frequently showing granules with abnormal sizes and shapes. Nuclear hyposegmentation, with coarse margination of chromatin associated with cytoplasm immaturity, also was observed. In addition to classic micromegakaryocytes, megakaryocytes with a decreased number of granules and demarcation membranes were present in most exposed patients.

Ultrastructural evidence of myelodysplasia involving multiple cell lineages was found in only 3 of 17 patients in the unexposed group. The same patients had evidence of myelodysplasia, as detected by light microscopic examination.

Immunophenotype. In general, immunophenotypes were in agreement with the cytologic classifications by the FAB criteria (Table 2).[21] In all patients in the exposed and unexposed groups, at least one of the myeloid markers (i.e., CD33, CD13) had positive results, whereas the CD14 monocyte-associated marker was found in patients with a monocytic component, classified as AML-M4 and AML-M5. Unequivocally positive results for the CD41 platelet antigen were found in a

Table 2. Immunologic Findings in Patients With AML Classified According to the FAB System (Bennett 1985): Exposed *Versus* Unexposed Subjects*

Immunologic markers	FAB subtype						
	M1	*M2*	*M3*	*M4*	*M5*	*M6*	*M7*
CD34							
Exposed	1/1	6/6	0/1	6/7	2/2	1/1	1/2
Unexposed	0/2	5/6	0/1	1/9	0/7	0/1	0/0
CD33							
Exposed	1/1	6/6	1/1	7/7	1/2	1/1	2/2
Unexposed	1/2	6/6	1/1	9/9	2/7	1/1	0/0
CD14							
Exposed	0/1	2/6	0/1	7/7	2/2	0/1	1/2
Unexposed	0/2	0/6	0/1	9/9	7/7	0/1	0/0
CD13							
Exposed	1/1	6/6	1/1	7/7	1/2	1/1	2/2
Unexposed	1/2	5/6	1/1	9/9	2/7	1/1	0/0
CD41a†							
Exposed	0/1	2/6	0/1	4/7	0/2	0/1	2/2
Unexposed	0/2	0/6	0/1	0/9	0/7	0/1	0/0
Lymphoid‡							
Exposed	0/1	0/6	0/1	3/7	1/2	0/1	0/2
Unexposed	0/2	0/6	0/1	1/9	0/7	0/1	0/0

FAB: French–American–British; AML: acute myeloid leukemia.

* Number of patients with a positive finding/number of patients tested.

† Patients with 5–10% unequivocally positive blasts (minor megakaryoblastic component) are classified as positive cases in this table. Only AML-M7 had more than 20% positive cells.

‡ Two patients had a positive finding for CD7 and three had a positive finding for CD10.

minority of cells (5–10%) in six patients categorized as exposed. Inappropriate expression of lymphoid antigens was detected in four exposed patients, three of whom had positive results for CD10 and one for CD7. Positive results for CD7 also were found in one unexposed patient.

The CD34 stem cell marker[22] was expressed in more than 20% of blast cells in 17 of 20 patients in the exposed group and 6 of 26 patients categorized as unexposed.

Cytogenetics

Detailed results in each category of patients are reported in Tables 3 and 4. Overall, clonal chromosome aberrations were detected in 15 of 18 patients exposed to pesticides, 7 of 7 patients exposed to organic solvents, and 10 of 34 patients classified as unexposed, based on anamnestic findings. Nine exposed patients (five to pesticides and four to organic solvents) had three or more events of translocation or nondisjunction in the same clone, thus fulfilling the definition of "major karyotype aberrations."[23] Recurring chromosome

Table 3. Clonal Chromosome Aberrations in 25 Patients With AML Categorized as Exposed

Patient no.	Age (yr)	FAB subtype	Sample	Karyotype	Abnormal cells/ normal cells
Pesticides (three patients had normal karyotypes)					
1	64	M7	PB	46,XY,del(7)(q22q35)/45,XY,del(7)(q22q35),−9	12/0
2	73	M7	BM	45,XY,−17	4/6
3	61	M2	BM	45,XY,−5,i(17q)	7/3
4	61	M4	BM	47,XY,+8	5/5
5	51	M4	BM	45,XX,−5,t(22;?)(q11;?)	6/6
6	73	M2	BM	46,XY,del(11)(q13q23)	8/4
7	74	M4	BM	46,XY,t(11;?)(p15;?)	2/10
8	69	M4	BM	46,XX,del(6)(q22)	14/6
9	67	M4	BM	46,XX,i(1q+)/46,XX,i(1q+),i(11q)	8/3
10	74	M4	PB	44,XY,del(5)(q11q34),−7,−14,t(16;?)(q22;?)	8/3
11	82	M1	BM	45,XY,t(1;7)(p14;p11),del(3)(p11p15),−5,+der(5)t(5;17)(q31;q11), −17,t(17;?)(p?;?)/same without the der(5)chromosome	10/0
12	50	M4	BM	45,XY,−6,+der(6)t(6;7)(p23;p14),−7,−16,+der(16)t(11;16) (q13;q22),−19,+M1/same without M1	10/0
13	69	M2	BM	47,XY,+M (E-size)	10/0
14	56	M2	BM	44,XY,−3,−5,t(15;?)(q21;?),i(21q)/45,XY,−3,t(15;?)	12/0
15	72	M2	BM	43,XY,−5,del(11)(q23),t(15;12;13)(p11;q13;q13),del(16)(q22),t(17;?) (p12;?),−18,−21	
Organic solvents					
16	70	M3	BM	47,XY,ins(14;2)(q23,q13q37),t(5;?)(q31;?),t(11;12)(p14;q12),+21	10/0
17	63	M5	BM	47,XY,+10	12/0
18	33	M6	BM	49,XY,+11,t(13;?)(p11;?),+M1,+M2/50,XY,+11,t(13;?)(p11;?), +M1,+M2,+M3	10/7
19	55	M2	BM	44,XX,del(5)(q13q33),del(16)(q22),t(17;?)(p12;?),−18,t(20;?)(p11;?), −21/45,XX,del(5)(q13q33),t(17;?)(p12;?),t(20;?)(p11;?),−18,−21,+M	10/0
20	35	M2		46,XY,t(6;9)(p23;q34)	10/0
21	40	M6	BM	45,XY,t(13;?)(q14;?),del(20)(q11q13),−8	12/5
22	74	M5	BM	47,XY,+21	10/0

AML: acute myeloid leukemia; FAB: French–American–British; BM: bone marrow; PB: peripheral blood.

aberrations in exposed patients were −5/5q− (eight patients), deletions or translocations of 17p (five patients), deletion or translocations of 16q22 (four patients), −7/ 7q− and trisomy of 21q (three patients), and trisomy of

11q (two patients). Breaks at bands 6p23, 7p14, 11p14–15, and 11q13 were found in two patients for each.

Ten of 33 unexposed patients had chromosome aberrations. Two patients had the t(15;17), two patients

Table 4. Clonal Chromosome Changes in 34 Patients With AML Categorized as Unexposed*

Patient no.	Age (yr)	FAB subtype	Sample	Karyotype (no. of cells)	Abnormal cells/ normal cells
1	47	M4	BM	47,XX,+4	6/4
2	68	M4	BM	46,XY,del(5)(q14q21)	5/8
3	16	M3	BM	46,XY,t(15;17)(q22;q12)/47,XY,t(15;17)(q22;q12),+22	10/0
4	25	M1	BM	45,XY,inv(3)(q21q26),−8/46,XY,inv(3)(q21q26),del(8q)	9/2
5	21	M3	BM	46,XX,t(15;17)(q22;q12)	10/0
6	62	M2	BM	46,XY,t(8;21)(q22;q12)	4/10
7	71	M2	BM	46,XX,−4,del(5)(q21q32),+10,−14,del(17p),+M	8/3
8	24	M2	BM	46,XY,del(3)(q21q26),dup(17)(q12—q14)	12/4
9	64	M2	BM	46,XX,del(6)(q22)/47,XX,del(6)(q22),+22	10/0
10	60	M4	BM	46,XX,t(6;?)(q15;?)	7/7

AML: acute myeloid leukemia; FAB: French–American–British; BM: bone marrow.
* Twenty-four patients had normal karyotypes.

had aberrations of the long arms of chromosomes 3 and 6, and two patients had a 5q- chromosome, one of whom had major karyotype aberrations.

Discussion

For AML, the correlation of a specific cytogenetic pattern and occupational exposure to certain substances was well documented in previous studies including patients exposed to organic solvents, petroleum products, and, less frequently, pesticides.[6,24] This report extends previous observations, contributing cytogenetic data and describing morphologic and immunologic features in a series including 25 exposed subjects, to better define the clinicopathologic picture of AML in this subset of patients. Unlike some of the previously reported series,[5,6,24] most of our patients were from rural areas; therefore, toxic exposure from industrial work was less frequent. Although we cannot exclude occasional contacts with myelotoxic agents in patients categorized as unexposed,[25] we tried to be very selective when including a patient in the exposed group. As a matter of fact, exposed patients in this series had been handling organic solvents or dissolving and spraying pesticides for prolonged periods, in the absence of effective protection measures. By contrast, unexposed patients had no evidence of contact with such substances.

Morphologic, immunologic, and cytogenetic studies in this series clearly document that AML in patients occupationally exposed to toxic substances may represent a distinct cytogenetic and cytologic entity, with some overlapping features with SL. Discussion will focus on the relevant characteristics of this subtype of AML.

Cytologic Characteristics and Immunophenotype

Morphologically, patients exposed to pesticides and those exposed to organic solvents had unequivocal signs of myelodysplasia involving multiple-cell lineages, whereas morphologic aberrations of the non–blast cell population were less frequent and almost invariably confined to a minority of cells in patients categorized as unexposed. The presence of multilineage involvement was confirmed at the ultrastructural level in our exposed patients, thus showing similarity to some of the cytologic features of SL, a disease commonly regarded as a panmyelosis.[4] Because identification of myelodysplasia in leukemic BM may be somewhat arbitrary, we classified our patients according to previously published criteria[11] for the diagnosis of AML with TMDS and found the features of TMDS in more

than 60% of patients in the exposed group. It is interesting that, in one patient attaining remission after chemotherapy, a classic myelodysplastic syndrome with the features of refractory anemia with excess of blasts preceded overt leukemia relapse, in line with the previously identified pattern of relapse in patients with AML with TMDS.[26] These findings seem to indicate that AML in patients occupationally exposed to certain substances frequently may be associated with a preclinical myelodysplastic phase, possibly resulting from the involvement of a multipotent hematopoietic progenitor retaining partial capability to differentiate along multilineage pathways.[27,28]

Immunologic data seem to strengthen this view, in that they show positivity for the CD34 stem cell marker in 17 of 20 exposed patients (85%), a figure significantly higher as compared with that found in our unexposed subjects and in previous reports of unselected AML cases.[29] Also, the presence of a minor megakaryoblastic component, as shown by positive results for the CD41 platelet antigen (5–10% of the blast cells) in 6 of 18 exposed subjects, seems to document the occurrence of multilineage differentiation of leukemic progenitors, a finding previously documented in SL and therapy-related myelodysplasia.[30,31]

Cytogenetics

In this series, 7 patients exposed to organic solvents and 18 patients exposed to pesticides showed a high frequency (22 of 25 cases) of clonal changes in BM cells, frequent involvement of the long arms of chromosome 5 and/or 7, and multiple aberrations in the same clone, with 9 patients fulfilling the definition of major karyotype aberrations. These data are in line with previous reports[5,6,32] and seem to fit well with the classic cytogenetic picture of SL,[33] a leukemia subtype in which the long arms of chromosomes 5 and 7 have been shown to be involved preferentially, often in the context of complex karyotypes. Also, in five of our exposed patients, the presence of aberrations leading to total or partial monosomy of 17p is of interest in this framework, the short arm of chromosome 17 having been added recently to the list of chromosomal sites nonrandomly involved in therapy-related leukemias.[34] In this series, 17p- was the sole aberration in one patient and was detected in all abnormal cells in four patients with multiple related clones, thus excluding that this abnormality may represent a late event in the cytogenetic evolution of highly abnormal cell lines. The same holds true for 6p rearrangements,[35,36] found in this series in two exposed patients. Thus, similar leukemogenic mechanisms may be operative in this subset of AML and in SL,

Figure 1. Frequencies (percentages) of involved chromosomes in 25 patients exposed to pesticides or organic solvents (dark columns) and 34 not exposed (light columns). Left side: Numerical changes; right side: structural aberrations.
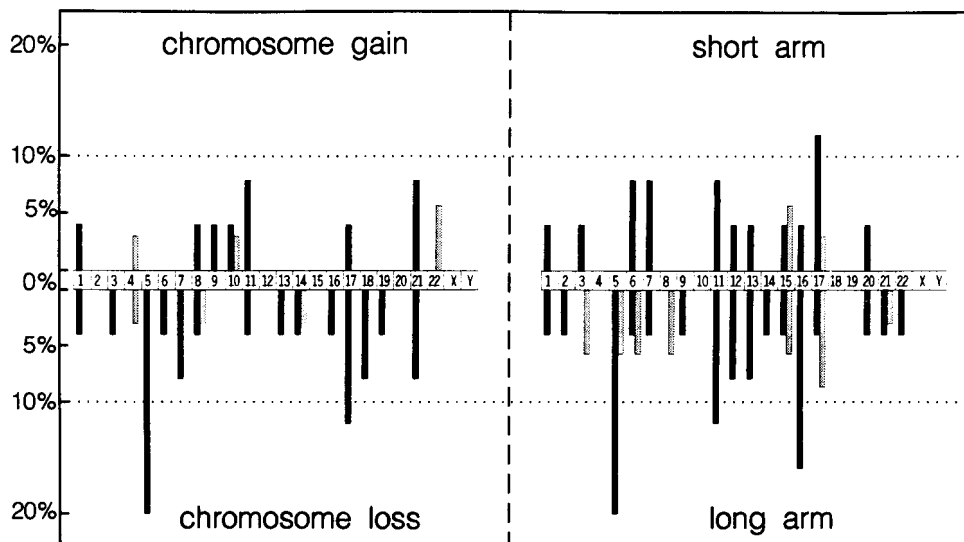
suggesting that specific chromosome regions may be the target of mutagenic compounds present in the environment and of cytotoxic drugs.

It is interesting that other recurring aberrations detected in our exposed patients, such as trisomy 11q, breaks of 7p, and deletions or translocations of 11q13, are found commonly in a spectrum of myeloid stem cell disorders, including SL,[37–39] and thus may be related to the transformation of an undifferentiated stem cell.[40] Although the most common AML-specific translocations (i.e., t[15;17] or t[8;21]) were found only among unexposed subjects, four exposed patients in this series showed translocations involving chromosome 16q22. Unlike the classic inv(16),[41] aberrations of 16q22 in our four patients were observed in the context of complex karyotypes and associated with trilineage myelodysplasia, in the absence of abnormal eosinophils. The association of 16q22 breaks and professional or iatrogenic exposure to certain substances has not been documented previously; however, the existence of clinicopathologic differences between AML with inv(16) and with del 16(q22) were emphasized recently, the latter chromosomal aberration having been found in AML preceded by MDS without BM eosinophilia.[42]

Although far more cases must be studied before a definite correlation can be established between chromosome pattern and environmental exposure, our data show that, besides abnormalities of 5q and 7q, a constellation of chromosome aberrations may be associated nonrandomly with AML after exposure to pesticides and organic solvents. Based on our findings, the percentage frequency of chromosomal involvement in patients with AML with occupational exposure to organic solvents and pesticides is summarized in Figure 1.

Conclusions

We have delineated the cytogenetic and clinicopathologic picture of AML in patients occupationally exposed to pesticides and organic solvents, and we have shown that clear-cut differences exist as compared with AML arising in patients classified as unexposed, based on anamnestic findings. Cytologic and cytogenetic findings in our exposed patients are similar to those found previously in SL, thus providing indirect evidence that, like cytotoxic drugs, pesticides and some organic solvents may play an etiologic role in a subset of cases of AML. According to our data, AML in exposed patients not only represents a cytologic and cytogenetic entity, but it also exhibits distinct clinical features as well. Indeed, percentages of BM blasts and leukocyte counts were low in the exposed group, a feature typical of AML with TMDS.[11] Also, the differences observed in terms of outcome of induction therapy and survival with respect to unexposed subjects are striking, especially if one considers that other clinical features[43] in our series, such as patient age, FAB classes, and leukocyte count, cannot account for such differences. Of course, because chromosome aberrations have been shown to represent independent prognostic factors in AML,[44] the association of chromosomal abnormalities, frequently of the major karyotype aberration type,[45] with occupational exposure may have influenced clinical outcome in our series, thus accounting for the differences observed in the two groups.

References

1. Coltman CA, Dahlberg S. Treatment-related leukemia. *N Engl J Med* 1990; 322:52–3.

2. Jacobs A. Benzene and leukemia. *Br J Haematol* 1989; 72:119–21.

3. Sandler DP, Collman GW. Cytogenetic and environmental factors in the etiology of the acute leukemias in adults. *Am J Epidemiol* 1987; 126:1017–32.

4. Vardiman JW, Coelho A, Golomb HM, Rowley J. Morphologic and cytochemical observation on the overt leukemic phase of therapy-related leukemia. *Am J Clin Pathol* 1983; 79:525–30.

5. Mitelman F, Brandt L, Nilsson PG. Relation among occupational exposure to potential mutagenic/carcinogenic agents, clinical findings and bone marrow chromosomes in acute nonlymphocytic leukemia. *Blood* 1978; 52:1229–37.

6. Golomb HM, Alimena G, Rowley JD, Vardiman JW, Testa JR, Sovik C. Correlation of occupation and karyotype in adults with acute nonlymphocytic leukemia. *Blood* 1982; 60:404–11.

7. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, et al. Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French–American–British Cooperative Group. *Ann Intern Med* 1985; 103:460–2.

8. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, et al. Criteria for the diagnosis of acute leukemia of the megakaryocytic lineage (M7): a report of the French–American–British Cooperative Group. *Ann Intern Med* 1985; 103: 460–2.

9. Shibata A, Bennett JM, Castoldi GL, Catovsky D, Flandrin G, Jaffe ES, et al. The International Committee for Standardization in Haematology (ICSH): recommended methods for cytological procedures in haematology. *Clin Lab Haematol* 1985; 7:55–74.

10. Farrow A, Jacobs A, West RR. Myelodysplasia, chemical exposure, and other environmental factors. *Leukemia* 1989; 3:33–5.

11. Brito-Babapulle F, Catovsky D, Galton DAG. Clinical and laboratory features of de novo acute myeloid leukemia with trilineage myelodysplasia. *Br J Haematol* 1987; 66:445–50.

12. Breton Gorius J, Vanhaeke D, Pryzwansky KB, Guichard J, Tabilio A, Vainchenker W, et al. Simultaneous detection of membrane markers with monoclonal antibodies and peroxidasic activities in leukemia: ultrastructural analysis using a new method of fixation preserving the platelet peroxidase. *Br J Haematol* 1984; 58:447–58.

13. Cordell JL, Falini B, Erber WN, Glosh AK, Abdulaziz Z, MacDonald S, et al. Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984; 32:219–29.

14. de la Maza M, Sanchez O. Simultaneous G and C banding of human chromosomes. *J Med Genet* 1976; 13:235–43.

15. ISCN. An international system for human cytogenetic nomenclature. In: Harnden DG, Klinger HP, editors. 1985.

16. Worthing CR, Walker SB. The pesticide manual. London: The British Corp Protection Council, 1991.

17. Nicholas AH, Van Den Berghe H. Sister chromatid exchange and pesticides, with emphasis on organophosphates. Sister chromatid exchange. New York: Alan R. Liss, 1982; 20:327–54.

18. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, et al. The French–American–British (FAB) Cooperative Group: Proposals for the classification of the myelodysplastic syndromes. *Br J Haematol* 1982; 51:189–99.

19. McKenna RW, Parkin JL, Foucar K, Brunning RD. Ultrastructural characteristics of therapy-related acute nonlymphocytic leukemia: evidence for a panmyelosis. *Cancer* 1981; 48:725–37.

20. Castoldi GL, Beutler E. Erythrocytes. In: Zucker-Franklin D, Greaves MF, Grossi CE, Marmont AM, editors. Atlas of blood cells: function and pathology. 2nd ed. Philadelphia: Lea and Febiger, 1988:97–156.

21. Neame PB, Soamboonsrup P, Browman GP, Meyer RM, Benger A, Wilson WEC, et al. Classifying acute leukemia by immunophenotyping: a combined FAB immunologic classification of AML. *Blood* 1986; 6:1355–62.

22. Katz FE, Tindle R, Sutherlands DR, Greaves MF. Identification of a membrane glycoprotein associated with haematopoietic progenitor cells. *Leuk Res* 1985; 9:191–8.

23. Sandberg AA. The chromosome of human cancer and leukemia. 2nd ed. New York: Elsevier North Holland, 1990:408–10.

24. Mitelman F, Nilsson PG, Brandt L, Alimena G, Gastaldi R, Dallapiccola B. Chromosome pattern, occupation, and clinical features in patients with acute non-lymphocytic leukemia. *Cancer Genet Cytogenet* 1981; 4:197–214.

25. Lawler SD, Summersgill BM, Clinck HM, McElwain TJ. Chromosomes leukemia and occupational exposure to leukemogenic agents. *Lancet* 1979; 1:853–4.

26. Brito-Babapulle F, Catovsky D, Galton DAG. Myelodysplastic relapse of de novo acute myeloid leukemia with trilineage myelodysplasia: a previously unrecognized correlation. *Br J Haematol* 1988; 68:411–5.

27. Jinnai I, Tomonaga M, Kuritama K, Matsuo T, Nonaga H, Amenomori T, et al. Dysmegakaryocytopoiesis in acute leukemias: its predominance in acute myelomonocytic (M4) leukemia and implication for poor response to chemotherapy. *Br J Haematol* 1987; 66:467–72.

28. Cuneo A, Mecucci C, Kerim S, Vandenberghe E, Dal Cin P, Van Orshoven A, et al. Multipotent stem cell involvement in megakaryoblastic leukemia: cytologic and cytogenetic evidence in 15 patients. *Blood* 1989; 74:1781–90.

29. Borowitz MJ, Gockerman JP, Moore JO, Civin CI, Page SO, Robertson J, et al. Clinicopathological and cytogenetic features of CD34 (My 10) positive acute nonlymphocytic leukemia. *Am J Clin Pathol* 1989; 91:265–70.

30. Tabilio A, Herrera A, D'Agais MF, Vainchenker W, Allard C, Clauvel JP, et al. Therapy related leukemia associated with myelofibrosis: blast cell characterization in six cases. *Cancer* 1984; 54:1382–91.

31. Third MIC Co-operative Study Group. Morphologic, immunologic and cytogenetic (MIC) working classification of the primary myelodysplastic syndromes and therapy-related myelodysplasias and leukemias. *Cancer Genet Cytogenet* 1988; 32:1–10.

32. Narod SA, Dubé IA. Occupational history and involvement of chromosomes 5 and 7 in acute nonlymphocytic leukemia. *Cancer Genet Cytogenet* 1989; 38:261–9.

33. Whang-Peng J, Young RC, Lee EC, Longo DL, Schecter GP, DeVita VT. Cytogenetic studies in patients with secondary leukemia/dysmyelopoietic syndrome after different treatment modalities. *Blood* 1988; 71:403–14.

34. Pedersen-Bjergaard J, Philip P, Larsen SO, Jensen G, Byrsting K. Chromosome aberrations and prognostic factors in therapy-related myelodysplasia and acute nonlymphocytic leukemia. *Blood* 1990; 76:1083–91.

35. Mecucci C, Michaux JL, Louwagie A, Boogaerts M, Van Den Berghe H. The short arm of chromosome 6 is nonrandomly rearranged in secondary myelodysplastic syndrome. *Cancer Genet Cytogenet* 1988; 31:147–55.

36. Cuneo A, Kerim S, Vandenberghe E, Van Orshoven A, Rodhain J, Bosly A, et al. Translocation t(6;9) occurring in acute myelofibrosis myelodysplastic syndrome, and acute nonlymphocytic leukemia suggests multipotent stem cell involvement. *Cancer Genet Cytogenet* 1989; 42:209–19.

37. Takasaki N, Kaneko Y, Maseki N, Sakurakai M. Trisomy 11 in chronic myelomonocytic leukemia: report of two cases and review of the literature. *Cancer Genet Cytogenet* 1988; 30:109–17.

38. Mecucci C, Van Orshoven A, Boogaerts M, Michaux JL, Van Den Berghe H. Characterization of deletion of chromosomes 7 short arm occurring as primary karyotypic anomaly in acute myeloid leukemia. *Br J Haematol* 1989; 71:13–22.

39. Mecucci C, Van Orshoven A, Vermaelen K, Michaux JL, Tricot G, Louwagie A, et al. 11q– chromosome is associated with abnormal iron stores in myelodysplastic syndromes. *Cancer Genet Cytogenet* 1987; 27:39–44.

40. Castoldi GL, Cuneo A, Tomasi P. Phenotype-related chromosome aberrations and stem cell involvement in acute myeloid leukemia. *Haematologica* 1989; 74:525–9.

41. LeBeau MM, Larson RA, Bitter MA, Vardiman JW, Golomb HM, Rowley JD. Association of an inversion of chromosome 16 with abnormal marrow eosinophils in acute myelomonocytic leukemia. *N Engl J Med* 1983; 309:630–6.

42. Ohyashiki K, Ohyashiky JO, Kondo M, Ito H, Toyama K. Chromosome change at 16q22 in nonlymphocytic leukemia: clinical implication on leukemia patients with inv(16) versus del(16). *Leukemia* 1988; 2:35–40.

43. Swirsky D, de Bastos M, Parish SE, Rees JKH. Features affecting outcome during remission induction of acute myeloid leukemia in 619 adult patients. *Br J Haematol* 1986; 64:435–53.

44. Yunis JJ, Brunning RD, Howe RB, Lobell M. High-resolution chromosomes as an independent prognostic indicator in adult acute nonlymphocytic leukemia. *N Engl J Med* 1984; 311:812–8.

45. Cuneo A, Van Orshoven A, Michaux JL, Boogaerts M, Louwagie A, Doyen C, et al. Morphologic, immunologic and cytogenetic studies in erythroleukaemia: evidence for multilineage involvement and identification of two distinct cytogenetic–clinicopathological types. *Br J Haematol* 1990; 75:346–54.