Early Antiviral Antibody Response After Immunization With Viral Oncolysate: A Powerful Prognostic Marker for Acute Myelogenous Leukemia Remission Patients

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Twenty-four acute myelogenous leukemia (AML) patients in first clinical remission received, as a part of their maintenance therapy, repeated injections of viral oncolysate (i.e., avian influenza virus-infected, formalin-inactivated, allogeneic leukemia cells). The anti-oncolysate-virus antibody responses after a single injection, tested by a radioimmunoassay, were in inverse correlation to the remission duration ($p \leq 0.01$). The 25% of patients with the lowest responses had a median remission duration of more than 36 mo, with no relapses within the first 18 mo. In contrast, the 75% of patients with higher responses had a median remission time of less than 5 mo, and more than 80% relapsed within 18 mo. Despite the small number of patients, these differences are highly significant ($p \leq 0.001$). Immunization of remission AML patients with viral oncolysate provides a powerful prognostic test. Most early relapses can be predicted, with a modest rate of false-positive and false-negative predictions.

USE OF MODERN combination chemotherapy has increased the remission rate achieved with acute myelogenous leukemia (AML) to 50%–80% in recent studies.[1-8] Of all patients who have achieved complete remission, 5%–25% remain in remission after 3–5 yr or longer.[9-14] However, more than half of all AML patients still relapse within the first 12–18 mo,[4,5,14-16] and the median remission duration has only marginally increased. Preventing early relapse is therefore a most urgent goal on the way to an ultimate cure of this disease, and methods to detect patients with high risk of early relapse are of crucial importance. Numerous prognostic criteria have been proposed,[13,17-21] but none of them individually is sufficient to influence the strength of maintenance therapy. Recently, however, it has been shown that reasonable prognostic predictions can be obtained by a combination of six prognostic factors.[14] Most of these factors are evaluated at the time of diagnosis, and thus, do not reflect eventual changes in individual prognosis that relate to differential responses to induction treatment.

No prognostic sign has been proposed for patients who have successfully completed induction therapy. However, a test differentiating at the time of first remission between patients prone to early relapse and prolonged remission could improve the subsequent treatment.

Inactivated viral oncolysate, although active in animal models,[22,23] has not been effective in prolonging remission time in AML.[24-26] Interestingly, prolonged treatment with oncolysate resulted in antibody responses directed against the viral component, which correlated inversely to remission lengths.[27] However, the hemagglutination inhibition (HAI) test used in those investigations was not sensitive enough to allow early individual prediction of prognosis. We now show that by using a radioimmunoassay (RIA), it is possible to predict early relapses after a single injection of viral oncolysate.

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MATERIALS AND METHODS

Treatment of AML Patients With Viral Oncolysate

In a randomized study conducted from 1974 to 1977 by the Swiss Group for Clinical Cancer Research (SAKK), 27 AML patients in their first remission were treated with viral oncolysate as a part of the maintenance therapy. The background, design, and the results of the study have been previously presented.[24-26] Patients achieving a complete first remission were randomly placed in two groups: one group received chemotherapy in cycles of 4 wk; the other group, in addition to the same chemotherapy, received injections of viral oncolysate on day 15 of each cycle. The viral oncolysate consisted of in vitro avian influenza virus (AIV) infected, formalin-treated, allogeneic leukemic myeloblasts. Maintenance treatment was started 4 wk after completion of the induction treatment. The first oncolysate injection was given 2 wk later.

Sera

Pretreatment sera (s_0) and the first sera obtained after the start of the oncolysate injections (s_1) were analyzed for anti-AIV antibodies. In two cases, s_0 sera were no longer available. In a third patient, the time interval between the oncolysate injection and s_1 was only 3 days, as opposed to more than 12 in all other cases. These 3 patients were therefore not included, and only 24 of the original 27 cases are presented here.

All sera were stored at −20°C. Before assay, sera were heat-inactivated (30 min at 57°C) and treated with neuraminidase (type X, Sigma Chemical Company, St. Louis, MO), according to standard procedures.[28]

Iodination of Protein A

Protein A (Pharmacia Fine Chemicals, Uppsala, Sweden) was iodinated with ^{125}I by the lactoperoxidase method, as described.[29]

Lactoperoxidase was from Worthington Biochemical Corporation (Freehold, NJ, USA).

Preparation of Virus-Coated Chicken Erythrocytes

Avian influenza virus (A/Turkey/England/63, Havl, Nav3, Langham strain; identical to that used in the oncolysate treatment) was grown in the allantoic cavity of chicken eggs.[30] Allantoic fluids with hemagglutinin titers of 512 and higher were pooled. Fresh washed chicken erythrocytes (CHEC) were added at a concentration of 4×10^7 cells/ml. The suspension was gently stirred for 2 hr at 4°C and then centrifuged at 800 *g* for 15 min. The virus-coated, sedimented cells (AIV-CHEC) were washed 3 times in ice-cold phosphate-buffered saline (PBS). Uncoated control erythrocytes (C-CHEC) were washed in the same way.

A reaction mixture containing 0.25% glutaraldehyde (electron microscopy grade, Merck, Darmstadt, West Germany) and AIV-CHEC or C-CHEC at 4×10^7 cells/ml in PBS was gently shaken at 4°C for 10 min and centrifuged at 800 *g* for 5 min. The sediments were washed 3 times with cold PBS and resuspended in 0.1% PBS complemented with bovine serum albumin (BSA-PBS) containing 0.02% NaAzide, again at 4×10^7 cells/ml. These cell suspensions could be kept for several weeks at 4°C without detectable change in their binding of anti-AIV antibodies.

Anti-AIV Antibody Radioimmunoassay

Flexible, V-bottomed Microtiter plates were pretreated with 4% BSA-PBS for 4 hr at room temperature to reduce nonspecific binding of protein to the plates. Fifty microliters of serum diluted 1:100 in 0.1% BSA-PBS was combined with 50 μl of fixed AIV-CHEC or fixed C-CHEC, respectively, in triplicate. S_0 and S_1 paired sera of each patient were assayed on the same plate. The plates were sealed and incubated for 14.5 hr at 4°C on a shaker, then centrifuged and washed 3 times using 200 μl of 0.1% BSA-PBS per well. Then, 0.15 μg ^{125}I-Protein A in 25 μl 0.1% BSA-PBS was added to each well. The plates were incubated 30 min at 4°C, then washed again 3 times. Utilizing a "hot wire" technique, the plates were cut into single wells and the radioactivity bound was determined. Counts per minute (cpm) from 2 independent assays were averaged and converted to nanograms Protein A. The anti-AIV activity of a serum was defined as (ng Protein A bound to AIV-CHEC) − (ng Protein A bound to C-CHEC).

Statistical Methods

The correlation of the antibody response and the remission time was tested by the rank correlation test of Spearman.[31] The distribution of the remission lengths in various low-response and high-response groups was analyzed by the rank-sum test of Mann and Whitney.[31] To compare the cumulated relapse risks of these groups, 2 × 2 table tests were used. We chose to use one-sided tests because the correlation of high antibody responses and short remissions was proven in our previous work.[27]

RESULTS

Early Antibody Response to AIV in Patients Treated With AIV-Oncolysate

Levels of anti-AIV antibodies, measured as nanograms Protein A specifically bound to AIV-CHEC, are shown in Table 1. All s_0 sera (column 3) were clearly positive, but antibody levels did not correlate with remission durations. Furthermore, the sera of two normals included in these experiments were similarly positive (1.78 ± 0.21, and, respectively, 2.27 ± 0.14 ng Protein A bound). The s_1 sera (column 4) showed higher antibody levels for patients with short remissions, but a significant correlation was not found ($p \leq 0.1$). In contrast, the remission times and the antibody responses (defined as the differences between the s_0 and s_1 sera, column 5) have a significant reverse correlation ($p \leq 0.01$; $r = -0.483$). As the time intervals between the s_0 and s_1 sera vary considerably (time interval, column 6), a slightly better correlation ($r = -0.514$) is obtained when these variations are taken into account (response/interval, column 7, and Fig. 1).

In order to determine the risk of relapse for individual patients, the prognostic power of the test, and the rate of false-positive and false-negative predictions, the antibody responses were classified in 3 deliberately set arrangements. In the first, the patients with the 25% lowest responses were compared to the 75% with higher responses. The combinations in the others were 33% versus 67%, and 50% versus 50%. The cumulated relapse-risks of these low-response/high-response groups were calculated from the data in Table 1 and are shown in Fig. 2. The low-response groups have a lower relapse risk than their counterparts. For the 25%/75% and the 33%/67% arrangements, the differences are significant up to 30 mo. None of the 25% lowest response patients relapsed during the first 18 mo, and at 30 mo, there was only 1 relapse. In contrast, 60%–70% of patients in all high-response groups relapsed within 6 mo, and 80% within 18 mo. The remission times of the same groups are shown in Fig. 3. The median remission time is less than 6 mo for all high-response groups. In contrast, it is more than 36 mo for the 25% lowest group and more than 30 mo for the other two low-response groups. The differences are significant for all three arrangements (50%/50%: $p \leq 0.05$; 33%/67%: $p \leq 0.025$; 25%/75%: $p \leq 0.001$). The data regarding the power of prediction of relapse and the rate of false-positive and false-negative relapse predictions are presented in Fig. 4. For the 75% high-response group, no relapses other than those predicted would occur during the first 18 mo, all relapses would be predictable (Fig. 4A). Consequently, there would be no false-negative predictions for the same period (Fig. 4C). Around 20% of the patients of this group, though predicted to relapse, would not relapse within 18 mo (false-positive predictions, Fig. 4B). With the 33%/67% and the 50%/50% arrangements, a smaller part of the relapses would be predicted (Fig. 4A), but with the same share of false-positive predictions (Fig. 4B) and more false-negative predictions (Fig. 4C). The 25%/75% arrangement therefore offers the most advantages.

Table 1. Antiviral Antibody Response After One Dose of Viral Oncolysate

Patient	Months of Remission	Anti-AIV Antibodies* in s_0	Anti-AIV Antibodies* in s_1	Antibody Response	Time Interval†	Response per Time Interval
1	72‡	1.97	2.04	0.07	1	0.07
2	63‡	2.46	1.73	−0.73	1.5	−0.49
3	55‡	1.55	3.15	1.60	1	1.60
4	49	2.10	2.63	0.53	3.5	0.15
5	40	2.91	2.10	−0.81	3.75	−0.22
6	35	1.98	1.52	−0.46	1	−0.46
7	31	1.49	0.93	−0.56	4	−0.14
8	31	2.13	2.52	0.39	1.25	0.31
9	19	1.65	1.42	−0.23	1.25	−0.18
10	15	2.38	3.27	0.89	2.75	0.32
11	12	2.27	3.92	1.65	2.5	0.66
12	9.6	3.63	6.82	3.19	2	1.60
13	7.5	2.16	2.29	0.13	1.25	0.10
14	5.7	1.44	4.03	2.59	1.25	2.07
15	5.2	3.87	5.83	1.96	1.5	1.31
16	4.6	1.09	1.45	0.36	1.5	0.24
17	4.5	3.79	4.72	0.93	3.5	0.27
18	3.3	3.75	3.95	0.20	1.25	0.16
19	2.8	1.53	1.78	0.25	3.25	0.08
20	2.8	0.75	1.32	0.57	1.25	0.46
21	2.5	3.00	3.52	0.52	1.5	0.35
22	2.3	2.21	4.72	2.51	1.25	2.01
23	2.3	1.36	3.27	1.91	1.25	1.53
24	1.0	1.47	2.84	1.37	1.25	1.10
Rank correlation, p		NS	<0.1	≤0.01		≤0.01
Correlation coefficient, r		−0.119	−0.336	−0.483		−0.514

Data of all patients, mean of two independent experiments.

*Nanograms Protein A bound to AIV-CHEC minus nanograms Protein A bound to C-CHEC.

†In units of 12 days, from date of oncolysate injection to date of s_1.

‡Continuing remission.

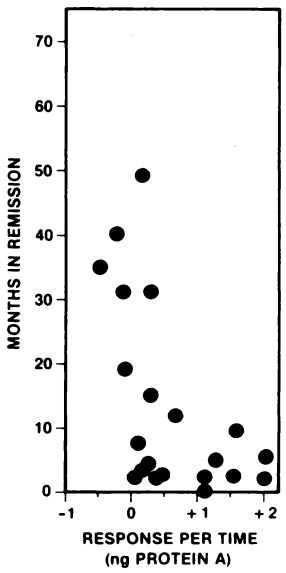

Fig. 1. Antiviral antibody responses per time interval and remission lengths are inversely correlated ($p < 0.01$; compare Table 1). (O) In continuing remission; (●) relapsed.

DISCUSSION

We report the correlation of the early antiviral antibody response of 24 AML remission patients immunized with viral oncolysate and the duration of their remissions (Table 1, Figs. 1 and 3). The risk of relapse of high responders is much higher than the risk of low responders (Fig. 2). Under optimally set conditions, i.e., if only the patients with the 25% lowest responses are defined as low responders, the test is very effective in detecting the patients prone to relapse within 18 mo (Fig. 4A), while carrying a modest rate of false-positive relapse predictions (Fig. 4B) and a negligible rate of false-negative predictions (Fig. 4C). The oncolysate injections have further been shown to be free of side effects and not to influence the course of the disease.[24-26] As the patients of this study were randomly selected to receive viral oncolysate, they form a representative sample of AML patients. Therefore, the findings made here retrospectively may be assumed to be typical for AML patients in general and, thus, may serve as the base for a truly predictive test.

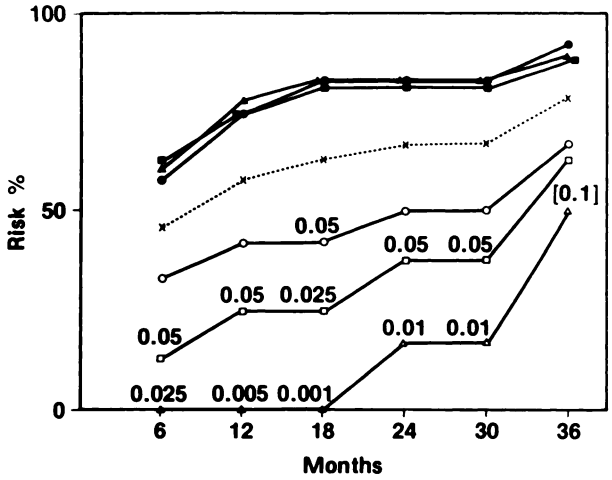

Fig. 2. Cumulated relapse risk of low-response and high-response groups. The figure shows the risk of patients of a certain response group to relapse within a certain time. Patients from all high-response groups (●, 50%; ■, 67%; ▲, 75% highest responses) have a 60%–70% risk to relapse within 6 mo, and a more than 90% risk to relapse within 36 mo. In contrast, the risk of patients belonging to the 25% lowest response group (△) is less than 10% at 18 mo, and still below 50% at 36 mo. The risks of the 33% lowest (□) and the 50% lowest (○) are intermediate. For comparison, the risk of the patients irrespective of their antibody response is indicated by the broken line. Numbers accompanying the low-response curves indicate the significance (p) at which the risk at the time indicated is different from the risk of the corresponding high-response group.

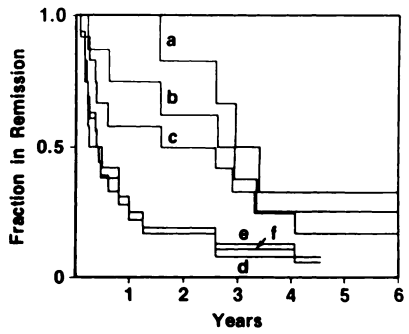

Fig. 3. Remission times of high-response and low-response groups. The median remission duration is less than 6 mo for all high-response groups (d, e, f), but more than 30 mo for all low-response groups (a, b, c). The differences are significant as follows: 25% lowest (a) versus 75% highest (d), $p \leq 0.001$; 33% (b) versus 67% (e), $p \leq 0.025$; 50% (c) versus 50% (f), $p \leq 0.05$.

In a previous report, we showed that, after prolonged immunization, titers of hemagglutination inhibiting antibodies in the sera of the same patients are of prognostic importance.[27] In comparison, the HAI tests and the newly developed RIA show a good correlation. When the ranks of antibody responses measured with RIA or HAI after 1 injection of oncolysate were compared to the ranks in the HAI test performed after 5 injections of oncolysate, the correlation was significant ($p \leq 0.01$; $r = 0.67$). The 2 patients with an antibody response after a single injection of oncolysate also detectable by HAI tests ranked highest in the RIA also (patients 14 and 22). This correlation is all the more remarkable because antibodies to any viral surface antigen will be detected by the RIA, while the HAI tests detects antibodies to a single antigen only—the viral hemagglutinin.

Because of the low sensitivity of the HAI test, 5 injections of viral oncolysate, 4 wk apart each, were needed before a prognostic difference became evident. Consequently, the HAI test is of little practical value. In contrast, injecting the oncolysate 2 wk after completion of the induction treatment, and using the RIA on sera obtained 2 wk after the immunization, yields prognostic predictions as early as 4 wk after induction

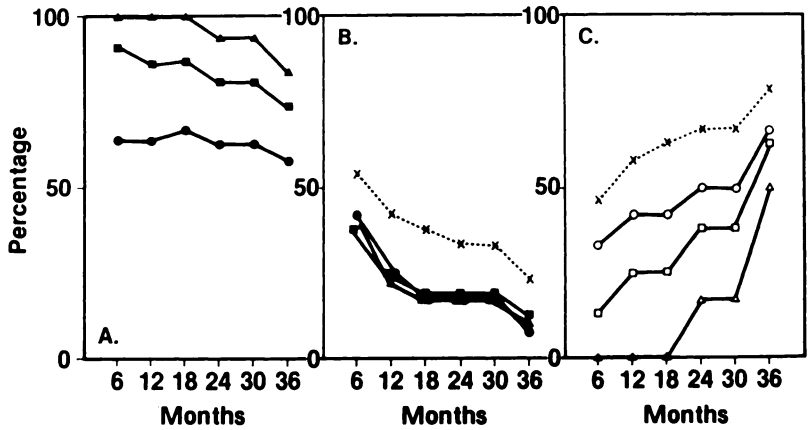

Fig. 4. Power and accuracy of prediction of relapse. (A) Percentage of relapses actually occurring within a certain time that can be predicted if the 75% highest (▲), 67% highest (■), or 50% highest (●) responses are defined as predicting relapse. (B) False-positive predictions. Percentage of patients not relapsing within a certain time, though, as high responders, they are predicted to do so. (X--X) False-positive predictions if 100% of the patients were predicted to relapse. Other symbols are as in A. (C) False-negative predictions. Percentage of patients relapsing within a certain time, though, as low responders, they are predicted not to relapse. (X--X) false-negative predictions if 100% of the patients are predicted not to relapse; (○) 50% lowest; (□) 33% lowest; (△) 25% lowest responses.

treatment. This test should therefore prove especially helpful to detect and prevent early relapses.

Surprisingly, all preimmunization sera, as well as the normal sera included in the tests, were positive for antiviral antibodies. It is possible that natural human antibodies to chicken erythrocytes are involved, or that antibodies against human influenza viruses cross-react with the virus used for the oncolysate. Cross-reactive antigens have been reported for a variety of human and animal influenza viruses, though not for the particular combination used here (AIV, strain A/Turkey, England/63, Langham strain, and any of the investigated human viruses) in HAI and neuraminidase inhibition studies.[32,33] However, there may still be similarities of these antigens with respect to epitopes not involved in hemagglutination or enzyme activity.

It remains uncertain whether the patients with a negative antibody response in the RIA had a real decrease of antibody titer, since no patient showed a significant (t test) decrease in all of the four repeated RIA tests.

The reasons for the correlation between antibody formation and remission duration also remain unclear. Very simply, it may be speculated that an efficient cytostatic treatment is acting both on the leukemia and the immune system, while insufficient therapy would allow both compartments to function. If this explanation is correct, immunization with the virus alone, or with an even simpler antigen, should give the same correlations. However, we observed in some patients considerable oscillations in sequential HAI titers that correlated closely to oscillations in the number of myeloblasts in bone marrow.[27] Sequential titers of antibodies to measle and rubeola virus did not vary. These findings suggest that other, more subtle and specific, immunoregulatory mechanisms lead to this correlation.

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REFERENCES

1. Bennet JM, Begg CB, Sartiano GC, Tartaglia A, Carbone PP: The chemotherapy of adult acute myeloid leukemia. Proc AACR/ASCO 21:434, 1980 (abstr)

2. Gale RP, Cline MJ: High remission-induction rate in acute myeloid leukemia. Lancet 1:497, 1979

3. Jacquillat CL, Weil M, Gemon-Auclerc MF, Izrael V, Gussel A, Boiron M, Bernard J: Clinical study of rubidazone (22 050 R.P.), a new daunorubicin-derived compound, in 170 patients with acute leukemias and other malignancies. Cancer 37:653, 1976

4. Keating MJ, Smith TL, Gehan EA, McCredie KB, Bodey GP, Spitzer G, Hersh E, Gutterman J, Freireich EJ: Factors related to length of complete remission in adult acute leukemia. Cancer 45:2017, 1980

5. Yates JW, Glidewell O, Wiernik P, Holland JF: A CALGB study of adriamycin vs. daunorubicin induction and a four vs. eight week maintenance in acute myelocytic leukemia. Proc AACR/ASCO 21:350, 1980 (abstr)

6. Peterson BA, Bloomfield CD, Bosl G, Gibbs B, Malloy M: Intensive five-drug combination chemotherapy for adult acute nonlymphocytic leukemia. Cancer 46:663, 1980

7. Rees JKH, Hayhoe FGJ: D.A.T. (daunorubicin, cytarabine, 6-thioguanine) in acute myeloid leukaemia. Lancet 1:1360, 1978

8. Uzuka Y, Liong SK, Yamagata S: Treatment of adult acute non-lymphoblastic leukemia using intermittent combination chemotherapy with daunomycin, cytosine arabinoside, 6-mercaptopurine and prednisolone-DCMP two step therapy. Tohuku J Exp Med 118(Suppl):217, 1976

9. Bodey GB, Freireich EJ, McCredie KB, Smith TL, Gehan EA, Gutterman JU, Hersh EM: Prolonged remissions in adults with acute leukemia following late intensification chemotherapy and immunotherapy. Cancer 47:1937, 1981

10. Coltman CA, Freireich EJ, Savage RA, Gehan EA: Long term survival of adults with acute leukemia. Proc AACR/ASCO 21:389, 1979 (abstr)

11. Ellison RR, Glidewell O: Improved survival in adults with acute myelocytic leukemia (AML). Proc AACR/ASCO 21:161, 1979 (abstr)

12. Keating MJ, Bodey GP, McCredie KB, Freireich EJ: Five year survival and remission duration in adult acute myelogenous leukemia (AML). Proc AACR/ASCO 20:416, 1979 (abstr)

13. Mertelsmann R, Thaler HT, To L, Gree TS, McKenzie S, Schauer P, Friedman A, Arlin Z, Cirrincione C, Clarkson B: Morphological classification, response to therapy, and survival in 263 adult patients with acute nonlymphoblastic leukemia. Blood 56:773, 1980

14. Peterson BA, Bloomfield CD: Long term disease-free survival in acute nonlymphocytic leukemia. Blood 57:1144, 1981

15. Clarkson BD: Current concepts of leukemia and results of recent treatment programs. Transplant Proc 10:157, 1978

16. Necheles TF: The acute leukemias. Monogr Hematol 1:84, 1979

17. Carpentier NA, Lange GJ, Fiere DM, Fournie GJ, Lambert PH, Miescher PA: Clinical relevance of circulating immune complexes in human leukemia: Association in acute leukemia of the presence of immune complexes with unfavorable prognosis. J Clin Invest 60:874, 1977

18. Ellison RR: Manifestations and prognostic features of acute myelocytic leukemia, in Terry WD, Windhorst D (eds): Immunotherapy of Cancer: Present Status of Trials in Man. New York, Raven, 1978, p 307

19. Fliedner TM, Perry S (eds): Advances in the Biosciences, vol 14: Workshop on Prognostic Factors in Human Acute Leukemia. Oxford, Pergamon, 1975

20. Sakurai M, Sandberg AA: Chromosomes and causation of human cancer and leukemia. XI. Correlation of karyotypes with clinical features of acute myeloblastic leukemia. Cancer 37:285, 1976

21. Spitzer G, Dicke KA, Gehan EA, Smith T, McCredie KB, Barlogie B, Freireich EJ: A simplified in vitro classification for prognosis in adult leukemia: The application of in vitro results in remission-predictive models. Blood 48:795, 1976

22. Lindenmann J, Klein PA: Increased immunogenicity of host cell antigen associated with influenza virus. J Exp Med 126:93, 1967

23. Lindenmann J: Viruses as immunological adjuvants in cancer. Biochim Biophys Acta 355:49, 1974

24. Sauter C, Cavalli F, Lindenmann J, Gmür JP, Berchtold W, Alberto P, Obrecht P, Senn HJ: Viral oncolysis: Its application in maintenance treatment of acute myelogenous leukemia, in Terry WD, Windhorst D (eds): Immunotherapy of Cancer: Present Status of Trials in Man. New York, Raven, 1978, p 355

25. Sauter C, Cavalli F, Lindenmann J, Gmür JP, Berchtold W, Alberto P, Obrecht P, Senn HJ: Viral oncolysis: Its application in maintenance treatment of acute myelogenous leukemia: Study analysis at 2.5 years, in Siegenthaler W, Luethy R (eds): Current Chemotherapy. Proceedings of the International Society of Chemotherapy. Washington DC, American Society for Microbiology, 1978, p 1112

26. Sauter C, Cavalli F, Lindenmann J, Gmür JP, Berchtold W, Alberto P, Obrecht P, Senn HJ: Viral oncolysis: Its applicaton in maintenance treatment of acute myelogenous leukemia. Study analysis at 3 years, in Rainer H (ed): Immunotherapy of Malignant Diseases. New York, F.K. Schattauer Verlag, 1978, p 397

27. Schuepbach J, Sauter C: Inverse correlation of antiviral antibody titers and the remission length in patients treated with viral oncolysate: A possible new prognostic sign in acute myelogenous leukemia. Cancer 48:1363, 1981

28. Grist NR, Ross CA, Gell EJ: Diagnostic Methods in Clinical Virology (ed 2). Oxford, Blackwell Scientific, 1974, p 103

29. Thorell JI, Johansson BG: Enzymatic iodination of polypeptides with 125-I to high specific activity. Biochim Biophys Acta 251:363, 1971

30. Blaskovic D, Styk B: Laboratory methods of virus transmission in multicellular organisms, in Maramorosch K, Koprowski H (eds): Methods in Virology, vol 1. New York, Academic, 1967, p 194

31. Diem K, Lentner C: Wissenschaftliche Tabellen Documenta Geigy. Basel, Geigy, 1968, p 123

32. Schild GC, Newman RW: Immunological relationships between the neuraminidase of human and animal influenza viruses. Bull WHO 41:437, 1969

33. Tumova B, Schild GC: Antigeneic relationships between type A influenza viruses of human, porcine, equine, and avian origin. Bull WHO 47:453, 1972