Third Symposium on New Aspects of Biology and Treatment of B Chronic Lymphocytic Leukemia

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IMAJ 2004;6:573–575

The third symposium on new aspects of biology and treatment of B chronic lymphocytic leukemia was held on 23 January 2004 at the Weizmann Institute, hosted by Professor Alain Berrebi (Hematology Institute, Kaplan Medical Center), Prof. A. Polliak (Leukemia Lymphoma Unit, Hadassah University Hospital, Jerusalem), and Dr. Idit Shachar (Weizmann Institute's Department of Immunology). Scientists from the Weizmann Institute and invited speakers from France, Poland, Germany and Israel discussed the latest developments in the field of CLL.

General overview

Although non-myeloablative allogenic stem cell transplantation and the benefits of the graft versus leukemia effect may increase the hope for cure of B-CLL, conventional chemotherapy or chemo-immunotherapy remain the key modalities to manage B-CLL patients. Medical oncologists and hematologists treating B-CLL patients have long understood that these patients could be separated into two groups: those who had a long history of stable disease and survive, and those who either presented with advanced symptoms or progressed rapidly to severe disease requiring therapy. We now know that reliable tests for cytogenetic aberrations (+12, 11q2 and 17p deletions), molecular markers (unmutated immunoglobulin variable region gene) and Zap70 expression characterize the poor prognostic form of the disease. This firmly supports the decision to treat these patients early, with the best and most effective drugs available, enabling a high response rate to initial therapy. This consists mainly of the purine analog fludarabine, while the adjunction of cyclophosphamide and the anti-CD20 monoclonal antibody rituximab seems to be a promising combination for achieving a high rate of molecular remission and prolonged survival, but cure remains doubtful.

Biology of B-CLL: new markers?

Dr. Michal Haran (Hematology Institute, Kaplan Medical Center) presented the results of a study performed in collaboration with Dr. Idit Shachar (Weizmann Institute). They found that Grb7, a non-catalytic intracellular adaptor protein involved in cell migration, is over-expressed in advanced-stage CLL, as compared to early-stage disease. A corresponding increase was observed in the *in vitro* migration of stage IV CLL cells compared to stage I cells. An interesting relationship between Grb7 and ZAP70, a marker defining the unmutated status and a poor prognostic factor, was also demonstrated. These findings show that Grb7 levels reflect the severity of disease, and may be used, in conjunction with ZAP70, to predict disease progression.

Rituximab-alliinase targeted allicin

Dr. Fabian Arditti and Prof. David Mirelman (Department. of Biological Chemistry, Weizmann Institute) developed a new application of the cytotoxicity of allicin as a novel approach in the treatment of B cell malignancies, based on the site-directed generation of allicin. Alliinase purified from garlic cloves was chemically conjugated with rituximab. The rituximab-alliinase conjugate bound target tumor cells and, upon addition of the substrate alliin, allicin molecules were produced *in situ*, effectively killing CD20+ tumor B cells. The *in vitro* results demonstrated that rituximab-alliinase targeted allicin induces apoptosis of the tumor cells. This was shown by the enhanced annexin binding at B-CLL cell surface 24 hours after treatment and the marked reduction in the number of B-CLL cells following 48 hours in culture. In comparison, treatment with the conjugate alone or with a conjugate consisting of a non-related monoclonal antibody caused only a minor and statistically non-significant anti-tumor effect. This combined non-toxic prodrug, alliin, with a potent cytotoxic product, allicin, and a targeting device, rituximab, might offer a more powerful and less toxic immunotherapy for B-CLL and other B cell malignancies.

Is B-CLL one or two diseases?

Prof. Guillaume Dighiero (Institute Pasteur, Paris) asked this provocative question. About one-third of patients with CLL never require treatment, have a long survival and die of causes unrelated to the disease. Most of these patients are stage A, express mutated VH genes and no deleterious chromosomal abnormality. In another third, an indolent phase is followed by disease progression. The majority of these patients belong to stage A, and may express unmutated V genes. The remaining third of patients have aggressive

CLL = chronic lymphocytic leukemia
B-CLL = B cell chronic lymphocytic leukemia

disease at the onset and need immediate treatment. Most of them express unmutated VH genes and frequently deleterious chromosomal abnormalities (11q and 17p deletion trisomy 12). Serum thymidine kinase and soluble CD23 levels are correlated to the mutational status of immunoglobulin genes. In agreement with previous reports indicating that, globally, immunoglobulin-mutated and unmutated cases have the same gene expression pattern, a supervised statistical analysis showed that only 85 genes were differentially expressed by a factor >2 between the two groups of CLL. As surrogate markers for immunoglobulin VH, *LPL, ZAP-70* and *SPG20* genes had a higher expression level among unmutated cases, whereas *ADAM29* and *NRIP1* genes were over-expressed in mutated patients. Further studies will assess the significance of these markers in the prognosis of CLL.

How does B-CLL fit with our current knowledge of B cell biology?

Dr. Ralf Kuppers (Cologne University) discussed this subject. B-CLL is a malignancy of mature B cells that express surface immunoglobulin. Most B-CLL co-express IgM and IgD, which was traditionally regarded as the hallmark of antigen-inexperienced naïve B cells. Moreover, nearly all cases of B-CLL are CD5-positive, a marker of B cells developing early in life and involved in the production of "natural" IgM. Therefore, it was assumed that B-CLL is derived from CD5-positive B cells.

Apart from B cell subsets in the peripheral blood, B cell populations detected in lymphoid tissue are also relevant for a discussion on the cellular origin of B-CLL cells. In this regard, marginal zone B cells appear to be of particular interest. A fraction of the cells are memory B cells, but marginal zone B cells are also the main B cell subset involved in T cell-independent immune responses type II. These cells appear to be partly responsible for the production of low affinity autoreactive antibodies, a feature shared with many cases of B-CLL. In conclusion, the unequivocal assignment of B-CLL cells to their normal counterpart is presently hampered not only by the puzzling observation that both mutated and unmutated B-CLL cases phenotypically resemble memory B cells, but also by the still ongoing discussion on the origin of human CD5-positive B cells, IgM-expressing B cells and marginal zone B cells.

Prof. Jacques Louis Binet honored

Prof. Aaron Polliack congratulated Prof. Binet, the esteemed host of the meeting, in celebration of his seventies. Prof. Binet, who has chaired the Department of Hematology at Hopital Pitie-La Salpetriere in Paris since 1969, specializes in B cells and chronic lymphocytic leukemia. In 1980 he described a staging system to determine prognosis based on multivariate analysis, stressing the number of involved lymphoid areas (A,B,C). The Binet classification is routinely used worldwide. As chairman of the French cooperative group on CLL, Prof. Binet initiated and directed several national studies and systemic trials on CLL therapy.

Prof. Polliack then revealed that in addition to his scientific achievements, Prof. Binet is also an artist. An expert in modern art, architecture and painting, he is a professor at the Ecole du Louvre and was appointed to acquire new paintings for French museums. Prof. Binet is also involved in the study of hospital architecture in France, and currently chairs the French Academy of Medicine. A bronze medal representing Jerusalem and an Israel-Museum copy of the Kumran manuscript were presented to Prof. Binet in recognition of his achievements.

Chemo-immunotherapy of CLL

On behalf of the German CLL study group, Prof. Michael Hallek (current Head of the Department of Hematology at Cologne) presented the new concept of treating CLL with the purine analogue fludarabine, an alkylating agent (cyclophosphamide) and the CD20 monoclonal antibody, rituximab. Previous studies performed by the German CLL Study group (Protocole CLL4) have already shown that the addition of cyclophosphamide to fludarabine increased the overall response to 94% and the complete remission to 20%, with a significant increase in the median overall survival to 47 months compared to 28 months with fludarabine alone. The Protocole CLL-8 will compare treatment with fludarabine and cyclophosphamide (FC) to fludarabine, cyclophosphamide and rituximab (FCR). Preliminary studies from MD Anderson Cancer Center and the CALGB group in the United States have shown a higher rate of molecular remission with the addition of rituximab to fludarabine and cyclophosphamide. Prof. Hallek announced that the Israeli Study group on CLL has been chosen as an independent center to recruit Israeli CLL patients for this study.

In conclusion, Prof. Hallek presented an interesting algorithm: "How to treat elderly advanced BCLL in 2004?" Since approximately 44% of CLL patients are at least 75 years old, the different groups were segregated as follows: Group I, called "Go-Go" and completely independent with no co-morbidity, will receive treatment as young adults with FC or FCR. Group II called "Slow-Go," with some impairment, will receive mild therapy, mainly chlorambucil. And group III "No-go," severely handicapped, will have palliative care only.

Allotransplant of stem cells with reduced intensity chemotherapy

On behalf of the Polish group, Prof. Alexander Skotnicki from Crakow presented the results of reduced intensity chemotherapy followed by allogenic stem cell transplant from HLA matched and unrelated donors in 24 advanced-stage CLL patients refractory to standard chemotherapy. This non-myeloablative conditioning regimen, including fludarabine, cyclophosphamide and monoclonal antibodies, induced a mixed chimerism in 54% and complete chimerism in 33% of the patients treated. Donor lymphocyte infusion converted complete chimerism in three patients. The results were remarkable, with 14 patients alive (58%) and a median follow-up of 22 weeks. Prof. Skotnicki emphasized the role of reduced intensity chemotherapy in the achievement of response in these severely affected CLL patients.

Ig = immunoglobulin

Allo- and autologous stem cell transplant: lessons from the EBMT registry

On behalf of the European Bone Marrow Transplantation registry, Prof. Mauricette Michallet (Head of the Bone Marrow Transplantation department at Lyon, France) reported the latest results of allo- and autologous stem cell transplants in CLL. In autotransplantation, a significant association was noted among the following factors: overall survival and disease-free survival, short interval from diagnosis to transplant, achievement of complete response before transplantation, and whole-body irradiation combining regimen; nonetheless, relapses occurred in 40% of the patients. A pilot study in 119 patients showed that 68% survived for 4 years after autotransplant, but cure remains doubtful. In contrast, cure of CLL after allotransplant has been documented (10 year leukemia-free survival) in 36% of patients transplanted with cells from identical sibling donors. The "mini allotransplant" EBMT survey has already recruited 72 patients and 70% received a conditioning regimen with fludarabine; 2 year event-free survival was noted in 60% of the patients; and 80% show more than 95% chimerism, emphasizing the reality of the graft versus leukemia effect.

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EBMT = European Bone Marrow Transplantation

English life, while very pleasant, is rather bland. I expected kindness and gentility and I found it, but there is such a thing as too much couth

S.J. Perelman (1903-79), American humorist and playwright

Capsule

Raji B cells, misidentified as THP-1 cells, stimulate HIV transmission

A number of studies examining interactions of dendritic cell (DC)-specific ICAM-3 grabbing nonintegrin (DC-SIGN) with viral pathogens have relied on monocytic transfectants as models for primary DCs. Li Wu et al. show that the presumed "THP-1" monocytic cells used in these studies are Raji B cells instead. Moreover, the authors demonstrate that true THP-1 cells do not support DC-SIGN-mediated HIV-1 transmission, whereas human B cell lines efficiently enhance this process. These data indicate that there are features common to B cells and DCs that facilitate transmission of HIV-1 and provide new insights toward the mechanism of DC-SIGN-mediated HIV-1 transmission.

Virology 2004;318:17

E. Israeli

Capsule

Peptide inhibitors of virus-cell fusion

Cooper et al. discussed the drug enfuvirtide as a case study in clinical discovery and development. The peptidic antiretroviral enfuvirtide (Fuzeon) is the first clinically approved antiviral fusion inhibitor and the first antiretroviral that must routinely be administered parenterally. Its extracellular activity results both in activity against current drug-resistant strains of HIV-1 and a low potential for systemic toxicities. As a peptide, enfuvirtide also exhibits few interactions with other antiretrovirals and concomitant medications used in HIV disease. Enfuvirtide shows potent antiretroviral activity and significantly improves medical outcomes in highly treatment-experienced patients with HIV-1 infection, but like other antiretrovirals must be given as part of a carefully selected combination regimen to minimize the risk of emergent drug resistance. Despite its subcutaneous route of administration, clinical data indicate that most patients can accept long-term enfuvirtide treatment with little difficulty or impact on daily activities. The only common adverse event associated with enfuvirtide use is injection-site reactions of generally mild-to-moderate severity, which are seldom treatment-limiting.

Lancet Infect Dis 2004;4:426

E. Israeli