UNIVERSITY (Ph. D.) THESIS

INVESTIGATION OF THE IMMUNE SYSTEM DURING THE TREATMENT OF MALIGNANT LYMPHOMA

Dr. Lajos Gergely

University of Debrecen
Medical and Health Science Center
Faculty of Medicine
Institute of Internal Medicine
3rd Department of Internal Medicine

Debrecen, 2005

UNIVERSITY (Ph.D.) THESIS

INVESTIGATION OF THE IMMUNE SYSTEM DURING THE TREATMENT OF MALIGNANT LYMPHOMA

DR. LAJOS GERGELY

TUTOR: PROF. DR. GYULA SZEGEDI

University of Debrecen
Medical and Health Science Center
Faculty of Medicine
Institute of Internal Medicine
3rd Department of Internal Medicine

Debrecen, 2005

1. Introduction

The malignat lymphoma was described by Thomas Hodgkin at the beginning of the 19th century. Since then we now many malignancies of the immune system different from the entity described by him. As the knowledge about malignant lymphomas increased during the last 20 years, several newer classification systems appeared. Nowadays we use the WHO classification outlined in 1997. This classification tries to use categories where the exact phenotype of the malignant cell is dominant. This can be achieved by surface phenotyping and genetic studies as well. However an important factor is that all the tests required to classify lymphomas should be greatly appliable and reproducible all over the world. The main reason for constant revision of classification systems is that clinicians want more knowledge from the pathologists regarding the behavior and prognosis of the disease. This is very important as several therapeuric options are available to choose from. However the mainstream for todays therapy is chemo- and radiotherapy which is toxic to the patient and bears potential carcinogen effect.We learned from more intensive therapies that the immediate succes and initial good results need to be paid for as late complications.The mainstream of therapy today should be personally adjusted, but in reality we could mostly asdminister the so called risk adopted therapy. The basic of this risk adopted therapy, that by using the histology this data is ammaneded with correct stage, prognostic factors like the international prognostic index. These together gide us what exact thjerapy is needed for best long term results with acceptable minimal toxicity. This also identify those patients who are potential candidates for high dose chemotherapy with autologous stem cell rescue. With all these we have a fairly good chance to succesfully treat the patient, however one can see that this line of evolution has reached its limits. Newer combination chemotherapy treatments are not proven to be superior to the old gold standard CHOP (cyclophosphamide, vincristin, adriamycin, prednisone).

Immunotherapy changed dramatically during the last few years. Anti-CD20 (rituximab) treatment is routinely used. However initial results with rituximab monotherapy have shown us, that the agent is not sufficinet if used alone, so we combine it with conventional chemotherpay with good results. Another routinely used agent is alemtuzumab (anti-CD52). The initial results are very promising with Y90 conjugated anti-CD20. This is superior to rituximab as it excerts its effect in pourly vascularized tissue and also my kill neighbouring tumor cells. Vaccination with tumor antigen alone or by the use of dendritic cells resulted in promising results, however this therapy awaits further results to confirm its effectiveness.

However still a physician treating lymphomas finds himself facing problems during the everyday work, as some patients do not respond to therapy as they should, and other achieve much better remission as predicted. This is only partially uanswered by todays genetic studies involving gene expression profiling and cluster analysis. There are only very few studies that focus not on the tumor, but on the self immune system, and its behaviour during the therapy. We are trying to perform some tests to get a closer view of these mechanisms.

2. Aim of the study

I presume that the immune system of lymphoma patients takes part in the fight against the malignant cells. I am tyring to analyze the steps involved in this process.

2.1. I show the survival data of non-Hodgkin lymphoma patients treated in the 3rd Dept. of Internal Medicine.

2.2. I investigate how cyclosporin A treatment affects B-CLL and associated paraneoplastic pemphigus.

2.3. I characterize IL-4 and IFN-gamma production of peripheral blood lymphocytes in non-Hodgkin lymphoma patients. I compare results of untreated patients with normal controls and patients in long term remission.

2.4. I measued antibodies against the extractable nuclear antigen (ENA) complex in non-Hodgkin lymphoma patients. This is trasnlated to the autoreactivity of the patients immune system. I try to correlate changes in autoantibody levels with response to initial treatment.

2.5. I measured antibodies against kardiolipin complex in the peripheral blood of non-Hodgkin lympohoma patients. I examine if there is a significant increase that predisposes these patients to thromboembolic disease. I also correlate results with response to treatment.

2.6. I report the succesful administration of anti-CD20 (rituximab), highlighting that the results are still not as good as they should be, and report on the changes of B-cells and activated T-cells during treatment in a case.

3. Patients and methods

3.1. We treated 238 non-Hodgkin lymphoma patients (115 female, 123 male) at the 3rd Dept. of Internal Medicine between January 1, 1996 and December 31, 2004. The mean age at diagnosis was 56 years (16 - 92 years). The patients were subdivided into agressive histological (n=170) and indolent (n=68) cases. The mean age at diagnosis was 52,3 years (16-92 years) fro agressive and 63,8 years (24-86 years) fro indolent lymphomas. Agressive lymphomas initially were either treated with CHJOP or with ProMACE-CytaBOM. Indolent lymphomas were treated with CVP, CHOP and chlorambucil for CLL.

3.2. I present the case of a 52 year old caucasian male, who was diagnosed with CD20+ small B-cell lymphoma in november 1998. In january 1999 the diagnosis was modified to B-CLL, according to caracteristic peripheral blood lymphocytosis. The patient developed a severe paraneoplastic pemphigus after one month of chemotherapy. High dose steroid was only partially succesful. We treated him with chlorambucil, CVP polichemotherapy, plasmapeheresis, cyclophosphamid, intravenous immunoglobulin with little success on the pemphigus. His CLL howeevere was well controlled, in partial remission during treatments. As the pemphigus persisted, and he developed severe side effects of long term steroid trreatment as well as bullae affecting both eyes, we decided to initate oral cyclosporin A with 7 mg/kg dose. Low dose steroid was used with cyclosporin for several months. Within 6 weeks the pemphigus resolved and the B-CLL was in complete remission. Continuous 36 month treatment maintained this good state for the patient.

3.3. We measured intracellular cytokine levels in 46 non-Hodgkin lymphoma patients (20 female and 26 male, mean age 56,4 /23-80/ yeras at diagnosis). Also 21 previously treated patients in long term (at least 12 months) remssion were included in the study (7 demale, 14 male, mean age 44,8 /19-74/ years). 22 healthy controls were used (9 female, 13 male, mean age 48,3 years /24-72/). Among the 21 patients in remission the 3 indolent lymphomas weere treated with CVP, and agressive lymphomas were either treated with CHOP for low and intermediate risk, and ProMACE-CyatBOM for high risk groups. Intracellular cytokine detection was performed with flow cytometry according to Jung's modified method {Jung, 1993 14 /id} {Aleksza, 2002 258 /id}.Briefly whole blood was collected into heparinized vacutainer tubes (Becton-Dickinson). We stimulated T cells in whole blood by phorbol 12-mistrate 13-acetate (PMA, 25 ng/ml, Sigma) and ionomycin (1 μg/ml, Sigma) for 4 hours at 37^{o}C in the presence of Brefeldin A 1μg/ml (Sigma). After the stimulation, the whole blood samples were stained to differentiate Th (T helper) and Tc (T cytotoxic) cells. Samples were separately incubated with quantum red-labeled anti-CD3, anti-CD4 or anti-CD8 (Sigma) mAb for 30 min., at room temperature, in dark. Then FACS Lysing Solution (Becton Dickinson) was used for 10 min. in order to lyse the erythrocytes and to fix the whole blood leukocytes. After a washing step (PBS, pH 7.4) the plasma membrane of the cells was permeabilized by the FACS Permeabilizing Solution (Becton Dickinson) for another 10 min. The fixed and permeabilized leukocytes were labeled by anti-IFN-γ-FITC and anti-IL-4-PE (Becton Dickinson) mAb for 30 min. at room temperature in dark. The samples were measured on a COULTER XL4 flow cytometer. Data of about 5,000 Th (CD4+) or Tc (CD8+) lymphocytes were collected in each sample. These cells were gated using the cytometer acquisition software based on their side scatter/forward scatter and

CD3, CD4 or CD8 positivity. The percentage of IFN-γ and IL-4 expressing cells was determined among all T cells (CD3+ positive cells). Intracellular cytokine production was measured separately in Th cells (CD4+ populations) and Tc (CD8+ population) T cells. As a quality control CD4+ and CD8+ cells should add up within +/- 10% of CD3+ cells. Statistical analysis was performed using SPSS software. Briefly the populations normal distribution was tested with Kolmogorov-Smirnov Z test, and in the case of normal distribution 2 independent sample T test was used. In case of non-normal distribution the Mann-Whitney statistical test was used. The significance level of p=0.05 was considered to be significant.

3.4. Anti-extractable nuclear antigen (anti-ENA) autoantibody levels were measured in non-Hodgkin lymphoma patients before therapy, during therapy and after the full 4-8 cycles of chemotherapy course. 66 patients were involved (36 males and 30 females), mean age at diagnosis was 54.98 years (24 to 80 years). 58 patients had high grade and 8 had low grade lymphoma. No patient had clinically evident polysystemic or organ specific autoimmune disease. The patients were divided into good responders (n=36) and bad responders (n=30) based on their therapeutic response to standard 4-8 cycle chemotherapy. Good responders all achieved complete response and maintained this state for at least a year. The rest of the patients were considered bad responders regarding the first line therapy. They either did not reach complete response, or needed additonal therapy to control the disease. 30 randomly selected age and sex matched normal controls were used from the laboratory database. Commercial ELISA kit (Hycor, Penicuik, UK) was used to measure anti-ENA antibody levels. The kit is a pan antibody screening test specific for anti-SSA (Ro), anti-SSB (La), anti-Scl70, anti-RNP, anti-Sm. We use this test in the laboratory as pre-screen, and in case of positivity the specificity of the antibody is measured. However in our cases no value above cutoff was measured, and we did not check for specificity of the measured antibody. Statistical analysis was done using the SPSS software.

3.5. Antibodies against the cardiolipin complex were measured in 31 non-Hodgkin lymphoma patients (16 female and 15 male). Mean age at diagnosis was 53,4 /20-80/ years. We measured anti-cardiolipin atnibodies with commercial ELISA kit (Orgentec Diagnostica GmbH, Mainz, Germany). The patients were divided into good and bad responders as described above. 20 randomly selected controls were used from the laboratory database where no malignancy or autoimmune disease was present.

3.6. I report our results with using the anti-CD20 (rtiuximab) monoclonal antibody to treat malignant lymphoma patients. The rituximab treatment was always administered by the companies recommendation. For monotherapy 375 mg/m^2 was given iv weekly for 4 weeks.

For combinations immuno-chemotherapy rituximab 375mg/m^2 iv. was given before each CHOP cycle (R-CHOP). The peripheral blood of patintes were anmalyzed for the presence and amount of lymphocyte subpopulations. Staining was perfromed with direct fluorochrom conjugated (FITC, PE and Quantum Red) monoclonal antibodies for human CD19 (Immunotech, Beckmand Couletr Inc CA, USA), CD3 and HLA-DR (Serotec, Oxford, England). Erythrocytes were lyzed, then samples washed with PBS and then fixed in 1% paraformaldehyde (Sigma). 5000 events' data was collected on the EPICS XL4 cytometer. B cells were detected based on CD19 expression, and activated T cell were detected as double positive for CD3 and HLA-DR.

4. Results

4.1. The 1. figure shows the overal survial of agressive lymphomas in our department. Close to 18% of the patients died within 12 months, and after 4 years the survival reaches a plateau with 60% of patients alive. This shows that we can cure 60% of all cses..

Figure 1. Overal survival in agressive non-Hodgkin lymphoma patients.

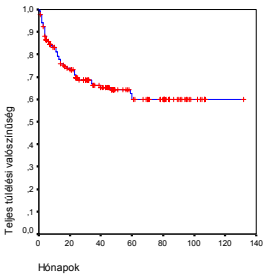


Figure 2. Overal survival in indolent non-Hodgkin lymphoma patients.

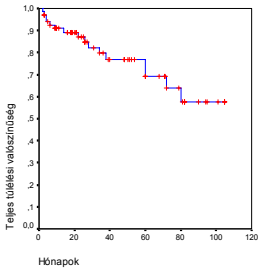


Figure 2. shows the overal survival for indolent lymphomas. One can see that 92% of the patients are alive 12 months after the diagnosis, which is a much better result than in agressive lymphomas. The curve did not ereach a plateau phase after 9 years, but still at that point 55% of patients are alive, which is slightrly worse than in agressive lymphomas.

4.2. We succesfully administered oral cyclosporin A for th treatment of B-CLL associated paraneoplastic pemphigus. The patient was continously treated for 36 months with good control of both diseases. No side-effects were observed. After discontinuation of cyclosporin A the B-CLL relapsed within 3 months, but the pemphigus did not reappear.

4.3. 46 untreated non-Hodgkin lymphoma patients' intracellular cytokine lvels were compared to 21 patients in remission for at least 12 months and to 22 healthy controls. Percentage values are within CD3+ lymphocytes, those cells that express either CD4 or CD8 and IL-4 and/or

IFN-gamma cytokines. Bold numbers indicate significant differences compared to the control group. Results are presented in table 1. The percentage of Th1 cells is significantly higher in untreated and remission patients compared to controls. The Th1 level in untreated patients is nearly similar to the value in remission patients. Untreated lymphoma patinets hacve a significantly higher Tc0 frequency compared to controls. During treatment this number decreased, but is still higher in remission patients, but this difference is statistically not significant.

Table 1.
Intracellularv cytokine production of peripheral blood lymphocytes in non-Hodgkin lymphoma

	Control (n=22)	Untreated patients (n=46)	Patients in remission (n=21)
CD4+ IFN-gamma (Th1)	21.83% (+/- 6.42)	*28.83% (+/- 10.68)*	*30.1% (+/- 12.32)*
CD4+ IL-4 (Th2)	1.19% (+/- 0.70)	*0.96% (+/- 0.9)*	*0.31% (+/- 0.28)*
CD4+ IL4/IFN-gamma (Th0)	0.51% (+/- 0.56)	0.54% (+/- 0.72)	0.6% (+/- 0.78)
CD8+ IFN-gamma (Tc1)	43.32% (+/- 8.8)	42.1% (+/- 19.42)	42.86% (+/- 13.78)
CD8+ Il-4 (Tc2)	0.62% (+/- 0.75)	1.02% (+/- 2.18)	0.51% (+/- 0.69)
CD8+ IL-4/IFN-gamma (Tc0)	0.47% (+/- 0.44)	*1.3% (+/- 1.1)*	0.82% (+/- 0.7)

4.4. Measured anti-ENA autoantibody is higher in all patient groups compared to controlls. Examining all patients together did not show any further change during treatment. Results are shown in table 2. Significantly (p=0,01) higher leveles are detected compared to controls. Elevated anti-ENA antibodies did not correlate with any sign of autoimmunity in our cases. The patients are the divided into good and bad responderes as described above. Anti-ENA level of the good responders is slightly lower than bad responders, but thi is not a statistically significant difference. An interesting tendency is observed during treatment as the value of good prognosis patients is constantly increasing as opposed to the bad responder group where a constant decrease could be detected. By the end of treatment a clear difference is found between the two groups which is not statistically significant (p=0,07).

Table 2.
Anti-ENA antibody in different groups of lymphoma patients during the treatment.

	Pretreatment anti-ENA antibody	Anti-ENA value during treatment	Anti-ENA value after treatment
Control group (n=30)	0,68 U/l *(± 0,4)*		
All patients (n=66)	1,85 U/l *(±0,94)*	1,90 U/l *(±0,95)*	1,81 U/l *(±0,85)*
Good responders (n=36)	1,77 U/l *(±0,60)*	2,07 U/l *(±0,61)*	2,10 U/l *(±0,73)*
Bad responders (n=30)	1,94 U/l *(±0,61)*	1,64 U/l *(±0,53)*	1,46 U/l *(±0,46)*

4.5. IgG isotype anti-cardiolipin antibody levels in untreated lymphoma patients were significantly higher compared to controls (6,72 U/l vs. 5,68 U/l, $p<0,05$). However by chmeotherapy treatment , the initially elevated values are reducing, and reaching significantly lower values compared to the control population (4,69 U6L, $p<0,05$). No additional decrease could be observed during the rest of the treatment, as post treatment vaslues are nearly identical (4, 61 U/l). IgM isotype autoantibodies are similarly higher before treatment (5,50 U/l vs. 3, 14 U/l, $p<0,05$) compared to controls. Two-three cycles of chemotherpay drastically reduces this elevated value to 3,40 U/l. By subdividing the patients into good (n=19) and bad (n=12) responders their data is separately analyzed as well. The statistical analyzis is of limited value because of the small patient numbers. IgG antibodies are slightly higher in good responder patients pretreatment (7,32 U6L vs 6,28 U/l) compared to the bad responders. This cahnges during treatment, but by the end of therapy a significant change is found between the two subgroups, as the bad responder patients have significantly higher amount of antibody compared to the others (5,95 U6L vs. 4,08 U/l, $p<0,05$). There was no change between the groups according to IgM izotype autoantibody levels.

4.6. The results of anti-CD20 (rituximab) immune therapy has been reported from several centers in eastern-hungary. 54 pateints with diffuse large B-cell lymphoma were treatted with R-CHOP protocol,. and their data is presented. As not all the patients were treated by us, I am not reporting the data , but highlighting an interesting case from our patients. I only want to focus on a small point from the paper. Alltogether 231 cycles of R-CHOP were administered, there were 16 cases with side eefects (7%), and only 8 cases (3,5%) with severe infection with G-CSF neeed. Only 5 patients (2,2%) had to be hospitalized for this reason. I am reporting a case of B cell lymphoma, where we continously monitored peripheral blood lymphocyte parameters during treatment. this patient was diagnosed with CD20+ follicular (grade II) B-cell non-Hodgkin lymphoma in January 2000. I am reporting the CD19 stained peripheral blood B-lymphocyte levels measured with flow cytometry on the 1. figure. NA represents not available

data on the figure. Figure 2. shows the CD3+ and HLA-DR+ activated T-cells from the same patient at the same timepoints.

Figure 1.
Peripheral blood CD19+ B-cell in a patient with non-Hodgkin lymphoma

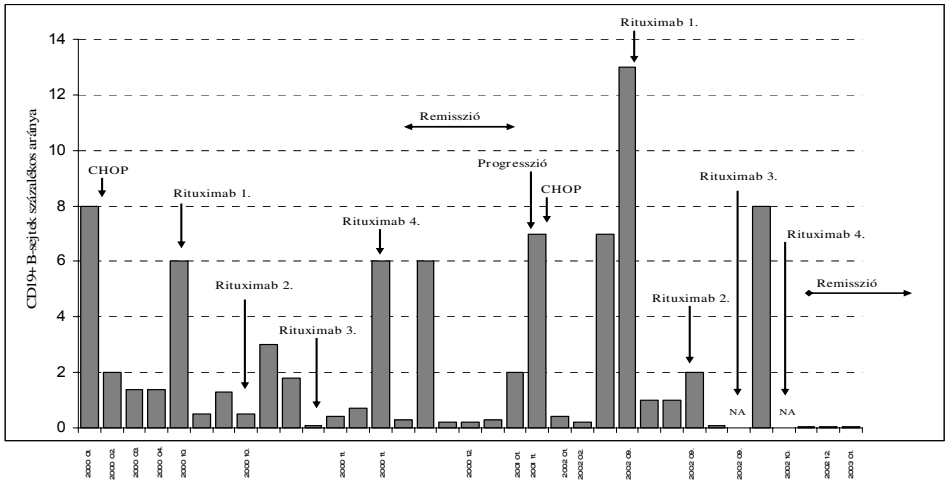
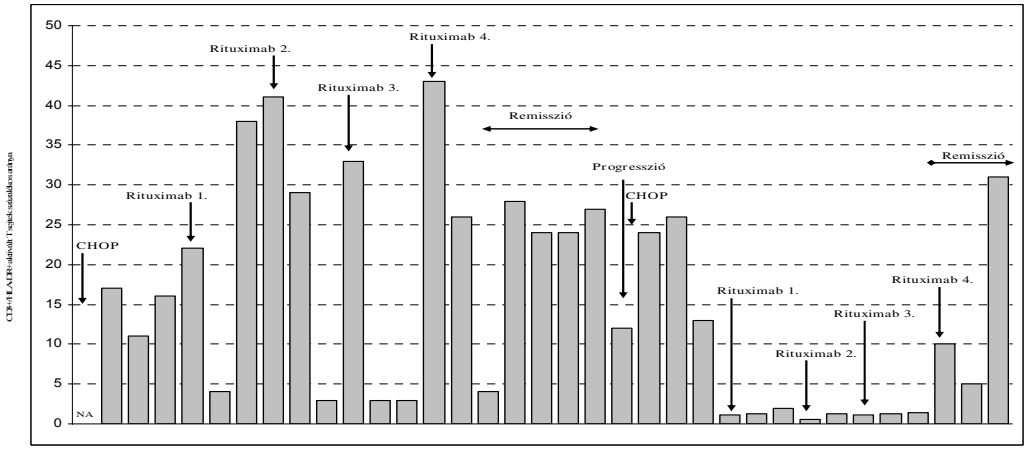


Figure 2.
CD3+ and HLA-DR+ circulating activated T-cells in the same patients peripheral blood.



By comparing the two figures interesting trends can be seen. It is demonstrated that an increase in activated T-cells is usually seen in cases where B-cell decrease is present. By administering treatment when elevated activated T-cell are present, this number is decreasing and an elevation in B-cell number is observed.

5. Megbeszélés

Our results demonstrate, that there are measurable changes in the immune system during lymphoma treatment. Survival data of our pateints is similar to what is described in the

literature, but we are unable to give accurate prognosis to all of our patients. The success with cyclosporin-A treatment highlights that by inhibiting the T-cells, and impairing T-B interactions, a B-cell controll is achieved. By measuring intracellular cytokines we demonstrated that there are measurable differences, with the dominant presence of type-1, IFN-gamma producing, cells. This may show an ineffective effort of the host immune system to eliminate the tumour. By observing this difference in remission patients as well, may explain the fact that these patinets usually have immune dysfunction, resulting even in autoimmune diseases in certain cases. The results with anti-ENA autoantibody measurements, raises that tehse patinets may have an increased autoreactivity, caused either by increased activation or impaired selection of these clones. As the antigen complex of ENA is only "visible to the immune system" during cell destruction it gives us a tool to measure immune response agains it for particular conditions. There is a similar concept with the anti-cardiolipin results, as these are also hidden atgignes, becoming "visible"during apoptosis when the negative charged parts are exposing themeselfs on the mebrane. By measuring reactivity against these antigens we can measure autoreactivity, and as a broder view reactivbity of the immune system. By following peripheral blood B and activated T cells the traetment schedule may not be optiomal for the patient.

6. Summary of the new scientific results:

1. Non-Hodgkins lymphoma patients treated at our department hav similar survival compared to the literature.
    a. 60% of agressive lymphoma patients are cured, about 4 years after the diagnosis.
    b. During the first year after diagnosis 18% of agressive lymphoma patients die.
    c. In diffuse large B-cell lymphoma 64% of patients are alive after 3 years, and this number did not decrease by furher years.
    d. Fivtifive percent of the indolent lymphoma patients are alive after 9 years, but the survival still not reaches a plateau phase.
    e. Mortality during the first year in indolent lymphomas is 8%.
2. B-CLL associated paraneoplastic pemphigus is succesfully treated with cyclosporin A. The continuous cyclosporin A treatment controlled B-CLL very well, which is a unique result. By discontinuing the cyclosporin-A after 36 months the B-CLL reoccured, but the pemphigus was cured.
3. By measuring intracellular cytokine prophile in 46 untreated and 21 remission non-Hodgkins' lymphoma patients the amount of Th0-1-2 and Tc0-1-2 cell precentages are measured.

a. Untreated lymphoma patients as well as patients in remission have a significantly ($p<0,05$) higher amount of Th1 cells than healthy controls. (28,85% és 30,1% vs. 21,83%).
b. Compared to the controls the amount of Th2 cells is significantly ($p<0,05$) lower in untreated and remission patients (0,96% és 0,31% vs. 1,19%)
c. Untreated patients have a significantly higher ($p=0,001$) percentage of Tc0 cells compared to controls (1,3% vs 0,47%).

4. We examined autoantibodies to extractable nuclear complex with ELISA in 66 non-Hodgkin lymphoma patients.
    a. I demonstrated the before, during and after chmeotherapy treatment the patients have significantly ($p<0,05$) higher amount of antibodies than controls (1,85 U/l, 1,80 U/l, 1,81 U/l vs. 0,68 U/l).
    b. By separately examining the godd responders (n=36) and bad responders (n=30) a non-significant ($p>0,05$) difference is found between them: a constantly increasing autoantibody level is found in good responders (1,77 U/l, 2.07 U/l, 2,10 U/l) and to the contrary a decreasing tendency is found in bad responders (1,94 U/l, 1,64 U/l, 1, 46 U/l).
5. Examining anti-cardiolipin autoantibodies in 31 non-Hodgkin lymphoma patients, the following is found:
    a. I could not detect a pathologically elevated value, causing thrombosis risk.
    b. Both IgG and IgM isotype autoantibodies are present at a higher concentration compared to controls. (IgG 6,72 U/l, IgM 5,50 U/l, control IgG 5,68 U/l, IgM 3,14 U/l).
    c. By treatment both isotype antibodies decrease to a close to normal value and did not change further.
    d. Separately examining the good responders (n=19) and the bad responders (n=12) the bad responders IgG isotype antibody decreases during treatment, but raises again by the end of chemotherapy (5,95 U/l).
6. I report a very desirable side-effect profile of rituximab.
    a. I report on a ptient, that the routinely and mechanically weekly administered rituximab monotherapy induces characteristic reactions. By monitoring the CD3+ and HLA-DR+ activated T-cells, and CD19+ B-cells the timing of therapy could be followed. I highlight that during therapy the self immune system shuold not be neglected.

In extenso papers covered ion the thesis:

1. Gergely L, Varoczy L, Vadasz G, et al. Successful treatment of B cell chronic lymphocytic leukemia-associated severe paraneoplastic pemphigus with cyclosporin A Acta Haematol - Basel 2003; 109 (4): 202-205 (IF: 1.874)

2. L. Gergely, M. Aleksza, L.Váróczy, A.Ponyi, S.Sipka, Á.Illés, Gy. Szegedi: Intracellular IL-4 / IFN-gamma Producing Peripheral T Lymphocyte Subsets in B Cell Non-Hodgkin Lymphoma Patients. Eur. J. Haematology. 2004 May; 72 (5): 336-41 (IF: 1.807)

3. Gergely L., Illés Á., Nagy Zs., Adamkovich N., Rejtő L., Szerafin L., Ujj Gy., Váróczy L., Radványi G., Borbényi Z., Varga Gy., Udvardy M.: Kezelési eredmények kombinált immuno-kemoterápiával diffúz nagy B sejtes nem-Hodgkin lymphomában. Magyar Belorvosi Archivum. 2004; 57 (1): 26-30

4. Gergely L., Illés Á.: Malignus lymphomák immunterápiája. Magyar Belorvosi Archivum. 2004; 57 (4): 59-67

5. L. Gergely, A. Dankó, I. Csípő, L. Váróczy, S. Sipka, M. Zeher, Á. Illés: Antibodies against extractable nuclear antigen (ENA) in non-Hodgkin lymphoma patients. Scand. J. Immunol. (közlésre elfogadva) (IF: 1.942)

Other publications:

1. L. Gergely, L. Cook, V. Agnello: A Simplified Method for Ca2+ Flux Measurement on Isolated Human B Cells That Uses Flow Cytometry. Clinical And Diagnostic Laboratory Immunology, 1997 Jan. (4): 70-74 (IF: 1.045)

2. Gergely L.: Cryoglobulinaemia és krónikus hepatítis C vírus fertőzés. Magyar Belorvosi Archivum, 1996 (4): 232-237

3. Illés Á., Vadász Gy., Gergely L., Szegedi Gy.: Hodgkin-kóros betegek kezelésével szerzett tapasztalataink. Magyar Belorvosi Archivum, 1998 (4): 283-288

4. Gyimesi E., Kiss A., Goda K., Bányai A., Kiss Cs., Telek B., Rácz K., Gergely L., Szegedi Gy., Sipka S.: Ciosztatikum-rezisztencia (multidrog-rezisztencia) vizsgálata citofluorimetriás funkcionális teszt alkalmazásával malignus hematológiai betegségekben. Magyar Belorvosi Archivum 1998. 51 (4): 301-305

5. Illés Á., Vadász Gy., Gergely L., Szegedi Gy.: Időskori Hodgkin kór. Magyar Onkológia, 1999 (43). 211-214

6. Matolcsy A, Borbényi Z, Demeter J, Egyed M, Fekete S, Földi J, Gergely L, Kajtár P, Kelényi G, Kiss A, László T, Lehoczky D, Losonczy H, Nagy M, Pál K, Pálóczy K, Radványi G, Semsei I, Varga G, Udvardy M: A minimális reziduális betegség kimutatása B-sejtes tumorok esetében az immunglobulin nehézlánc génre specifikus polimeráz láncreakció segítségével. Orvosi Hetilap, 2000 Jun 18; 141(25). 1403-6.

7. Á. Illés, G. Vadász, L. Gergely and G. Szegedi: Hodkin's disease in the elderly: A single institution retrospective study of 40 patients aged 65 or over. Hematologia, 2000 (30), 263-271 (IF: 0,405)

8. Illés Á., Gergely L., András Cs., Miltényi Zs., Szegedi Gy.: Hodgkin-kóros betegek hypothyreosisa. Magyar Onkológia 2001 45(5), 411-415

9. Varoczy L, Gergely L, Zeher M, Szegedi Gy, Illes A: Malignant lymphoma-associated autoimmune diseases - a descriptive epidemiological study. Rheumatol Int. 2002 Nov; 22 (6): 233-7. (IF: 1,0)

10. Varoczy L, Gergely L, Szakall Sz, Illes A: Angiocentric lymphomatoid granulomatosis and severe hypogammaglobulinaemia. Haematologia 2002;109(4): 535-41 (IF: 0,293)

11. Illés Á., Keresztes K., Miltényi Zs., Váróczy L., Olvasztó S., Redl P., Gergely L., Dankó K.: Hodgkin-kóros beteg kezelésének szokatlan késői szövődményei. Magyar Belorvosi Archivum 2002 55, 105-109

12. Miltényi Z, Gergely L, Illés Á: Hodgkin kóros betegek pericarditise. Orvosi Hetilap. 2002 Dec 1; 143 (48): 2687-9

13. Á. Illés, L. Gergely, Zs. Miltényi, K. Keresztes, S. Olvasztó, P. Redl, K. Dankó: Rare, late complications in a patient with Hodgkin's disease. Haematologia 2002 32(4) 509-518 (IF: 0,293)

14. Varoczy L, Gergely L, Illes A: Diagnostics and treatment of pulmonary BALT lymphoma: a report on four cases. Ann Hematol 2003 JUN 82 (6): 363-366 (IF: 1,241)

15. Váróczy L., Miltényi Zs., Keresztes K., Gergely L., Remenyik É., Illés Á.: Malignus kórképek halmozódása krónikus lymphoid leukemiában szenvedő betegünknél. Magyar Belorvosi Archivum 2003 56 (3): 127-130

16. Constantin T., Ponyi A., Garami M., Gergely L., Fekete G., Dankó K.: A juvenilis dermatomyositis klinikai sajátosságai. Orvosi Hetilap. 2003 Jun. 22; 144(25): 1245-50

17. Constantin T., Ponyi A., Bense T., Sallai Á., Gergely L., Fekete Gy., Dankó K.: A juvenilis dermatomyositis klinikai sokszínűsége – esetismertetés és irodalmi összefoglaló. Gyermekgyógyászat. 2004; 55(1)

18. Váncsa A., Ponyi A., Constantin T., Gergely L., Dankó K.: Dermatomyositishez társuló, késői megjelenésű extranodális follicularis lymphoma. LAM 2004; 14(2): 139-142

19. Ponyi A, Borgulya G, Constantin T, Vancsa A, Gergely L, Danko K.: Functional outcome and quality of life in adult patients with idiopathic inflammatory myositis. Rheumatology (Oxford). 2004 Sep 20 (IF: 3,76)

20. Váróczy L., Gergely L., Miltényi Zs., Illés Á.: Tüdő BALT-lymphoma kezelésével és követésével szerzett tapasztalatok. Magyar Belorvosi Archivum. 2004, 57(2): 83-87

21. L. Váróczy, L. Gergely, Zs. Miltényi, M. Aleksza, Á. Illés: Can CD3+/HLA-DR+ activated T cells predict the prognosis of non-Hodgkin's lymphoma patients ? Immunology Letters 2005; (97): 155-157 (IF: 1,710)

22. Keresztes K., Aleksza M., Baráth S., Miltényi Zs., Váróczy L., Gergely L., Sipka S., Illés Á.: *Helicobacter pylori*-fertőzés Hodkin-kóros betegeken. Hematológia Transzfuziológia 2004; 37: 265-71

23. Váróczy L., Krenács L., Gergely L., Bassam A., Illés Á.: Ritka, lymphadenomegálival járó kórképek. Hematológia Transzfuziológia 2004; 37: 248-256

24. Váróczy L., Gergely L., Aleksza M., Miltényi Zs., Illés Á.: Aktivált T-sejtek vizsgálata non-Hodgkin-lymphomás betegek esetében. Magyar Immunológia 2004; 3 (4): 35-39

Chapters:

1. KLINIKAI IMMUNOLÓGIA. Szerkesztette: Petrányi Győző, Dobozy Attila, Gergely Péter, Pálóczi Katalin, Szegedi Gyula, Szemere Pál. Medicina Könyvkiadó Rt. 2000. Ifj. Gergely Lajos, Gyimesi Edit, Antal-Szalmás Péter: Áramlási citometria alkalmazása a klinikai immunológiai laboratóriumi gyakorlatban. (könyvfejezet) 887-892

2. LEUKOCYTE TYPING VI. Editors: Tadamitsu Kishimoto, Hitoshi Kikutami et. Al. Garland Publishing Inc. 1997
    a. G. Lakos, E. Kiss, P. Soltesz, A. Kiss, E. Gyimesi, L. Gergely, Gy. Szegedi: PL15.1 Platelet clinical studies: Reactivity of Platelet monoclonal antibody panel with platelets of patients with antiphospholipid syndrome. P 686-687
    b. M. Zeher, Á. Olajos, L. Gergely, G. Lakos, Gy. Szegedi: CR25.4 Cytokine Receptor clinical studies: Dynamics of cytokine receptor expression in Sjögren's syndrome. P922-925
    c. E. Gyimesi, E. Kiss, L. Gergely, Gy. Szegedi: MC22.6 Myeloid Blind Panel flow cytometric analysis: Reactivity of Myeloid monoclonal antibodies with polymorphonuclear leucocytes and monocytes of patients with systemic lupus erythematosus and modulation of the binding by *in vivo* granulocyte-colony stimulating factor treatment. P1057-1059

3. Illés Á, Gergely L: Malignus lymphomák. In: Klinikai onkológia a gyakorlatban. Szerk: Szántó J, Medicina, 2005, pp 387-391.

4. Gergely L: Non-Hodgkin lymphomák. In: Klinikai onkológia a gyakorlatban. Szerk: Szántó J, Medicina, 2005, pp 391-407.

Abstracts:

1. O'Riordan DM, Gergely L, Deng N, et al.: Cytokine Messenger-RNA Expressionin Acute Pulmonary Infection. Am Rev Respir Dis 1993 Apr. 147 (4): A467-A467 Suppl. S

2. Gergely L., Illés Á., Vadász Gy., Szegedi Gy.: Melanoma malignum után jelentkező rapid progressziójú kifejezett malignitású B-sejtes non-Hodgkin lymphoma. Magyar Belorvosi Archivum 1997. 50(S1); 45

3. Vadász Gy., Illés Á., Nemes Z., Gergely L., Szegedi Gy.: Lethalis midline granuloma. Magyar Belorvosi Archivum 1997. 50(S1); 45

4. Antal-Szalmas P, Aleksza M, Gergely L, et al.: Intracellular cytokine determinations for assessment of Th1/Th2 distribution in autoimmune disorders. Cytometry, 2000 Apr 15. 42 (2): 133-134

5. Aleksza M, Antal-Szalmas P, Gergely L, et al.:Th1/Th2 like phenotype determinations in patients suffering from different autoimmune disorders
Cytometry, 2000 Apr 15. 42 (2): 142-142

6. Szomják E., Soltész P., Váróczy L., Gergely L., Veres K., Szabó Z., Szegedi Gy.: HIV-asszociált pneumonia miatt kezelt két esetünk diagnosztikus és terápiás tanulságai. Magyar Belorvosi Archivum 2000. 53(S2): 47

7. Váróczy L., Illés Á., Gergely L., Vadász Gy., Szegedi Gy.: Rapidan progrediáló T-sejtes anaplasticus lymphoma – esetismertetés. Magyar Belorvosi Archivum 2000. 53(S2): 53

8. Gergely L., Illés Á., Vadász Gy., Váróczy L., Kiss E., Zeher M., Szegedi Gy.: Lymphadenopathia, autoimmun betegség és lymphoma. Magyar Belorvosi Archivum, 2000. 53(S3): 80

9. Szomor Á., Molnár L., Iványi J., Radványi G., Nagy Zs., Karádi Á., Gergely L., Bányai A., Demeter J., Aryan H., Gasztonyi Z., Kiss A., Kollár B., Egyed M., Losonczy H., Kelényi G., Kereskai L., Pajor L.: Extranodalis érintettséggel járó és nodalis anaplasiás nagy sejtes lymphoma (ALCL) klinikopathológiai összehasnolítása. Magyar Belorvosi Archivum, 2000. 53(S3): 149

10. Illés Á., Redl P., Gergely L., Olvasztó S., Koszta Gy., Szegedi Gy.: Hodgkin-kóros beteg ritka késői szövődményei. Magyar Belorvosi Archivum 2001. 54(S1): 20

11. Gergely L., Váróczy L., Vadász Gy., Illás Á., Remenyik É., Szegedi Gy.: Non-Hodgkin lymphomához társuló paraneoplasticus pemphigus. Magyar Belorvosi Archivum 2001. 54(S1): 62

12. Váróczy L., Gergely L., Vadász Gy., Illés Á.: Tüdő (BALT-) –lymphoma diagnosztikája és kezelése – tapasztalataink három eset kapcsán. Magyar Belorvosi Archivum 2001. 54(S1): 81

13. Á.Szomor, L.Molnár, J.Iványi, G.Radványi, Zs.Nagy, Á.Karádi, L.Gergely, A.Bányai, J.Demeter, H.Aryan, Z.Gasztonyi, A.Kiss, B.KOllár, M.Egyed, H.Losonczy, G.Kelényi, L.Pajor: Extranodal involvement in primary systemic anaplastic large cell lymphoma (ALCL) in adults. /Abstract/ Annals of Hematology 2001 Suppl. III Vol. 80. pB144

14. Miltenyi Z, Illes A, Gergely L, Miltenyi L: Epidural involvement in Hodgkin's disease. Ann Oncol, 2002, 2 suppl: 114

15. L.Varoczy, L.Gergely, M.Zeher,G.Szegedi & A.Illes: Malignant Lymphoma-Associated Autoimmune Diseases – A Descriptive Epidemiological Study /Abstract/ Scand. J.Immunol. 2002 55, p527

16. Brúgós B., Szűcs G., Gergely L., Bodolay E., Szegedi Gy., Zeher M.: Felnőttkori hypogammaglobulinaemiás esetek. Magyar Belorvosi Archvum 2002. 55(S2): 39

17. Gergely L., Váróczy L., Aleksza M., Vadász Gy., Szász R., Illés Á.: Intracelluláris cytokinprofil vizsgálata non-Hodgkin lymphomás betegeinkben. Magyar Belorvosi Archivum 2003. 56(S1): 15

18. Rejtő L., Telek B., Kiss A., Batár P., Gergely L., Váróczy L., Udvardy M.: CD20-ellenes monoklonális antitest-kezeléssel szerzett tapasztalataink. Magyar Belorvosi Archivum 2003. 56(S1): 31

19. Vadász Gy., Simon Zs., Bereczky Zs., Hevessy Zs., Gergely L., Illés Á.: Gátlótest-haemophilia egy eset kapcsán. Magyar Belorvosi Archivum 2003. 56(S1): 38

20. Váróczy L., Gergely L., Aleksza M., Vadász Gy., Simon Zs., Batár P., Illés Á.: Aktivált T-sejtek vizsgálata non-Hodgkin lymphomás betegeinknél. Magyar Belorvosi Archivum 2003. 56(S1): 70

21. Gergely L., Váróczy L., Vadász Gy., Keresztes K., Illés Á.: Non-Hodgkin limfómás betegeink kezelési eredményei 1997-2003 között. Magyar Onkológia 2004. 48(S1); 6

22. Gergely L., Illés Á, Nagy Zs., Adamkovich N., Rejtő L., Szerafin L., Ujj Gy., Váróczy L., Radványi G., Borbényi Z., Varga Gy., Udvardy M.: Kezelési eredmények kombinált immuno-kemoterápiával diffúz nagy B-sejtes nem-Hodgkin lymphomában. Magyar Onkológia 2004. 48(S1): 17

23. Váróczy L., Gergely L., Miltényi Zs., Simon Zs., Illés Á.: Hospitalizálandó infekciók kemoterápiával kezelt non-Hodgkin lymphomás betegeinknél. Magyar Onkológia 2004. 48(S1): 17

24. L. Varoczy, L. Gergely, M. Aleksza, G. Vadasz, Z. Simon, A. Illes: Investigation of Activated T-Cells in Non-Hodgkin Lymphoma Patients. The Hematology Journal. 2004 (5) Suppl. 2., S188

25. L Gergely, A Danko, I Csipo, L Varoczy, A Illes, S Sipka, M Zeher: Antibodies Against Extractable Nuclear Antigen (ENA) in Non-Hodgkin Lymphoma Patients. Clin. Invest. Med. 2004. 27(4): 181D

26. Nagy G, Varoczy L, Dobrosi N, Gergely L, Szegedi C: Monitoring the intracellular calcium concentrations of lymphocytes during the treatment of malignant lymphomas. Tissue Antigens. 2004; 64 (4): 366-366

Cumulative impact factor of publicaítions (without abstracts): 15.37