

Mapping of B-cell epitopes in *E. coli* asparaginase II, an enzyme used in leukemia treatment

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Abstract

The enzyme L-asparaginase is a crucial component in the treatment of acute lymphoblastic leukemia (ALL). As all asparaginases in clinical use are derived from microorganisms, immunological reactions are the most important adverse events associated with asparaginase treatment. Two different methods, phage display and the SPOTs method, were used for the determination of clinically relevant epitopes. Comparison of the results showed that essentially the same domains were identified by the two methods, and thus ascertainment of relevant epitopes can be assumed. Determination of the specificity of the epitopes will be performed with serum from patients with different modes of immunological reactions and from individuals without evidence of an immune response after asparaginase administration.

Keywords: acute lymphoblastic leukemia; anti-asparaginase antibodies; epitope mapping; phage display; SPOTs.

Introduction

L-Asparaginase (EC 3.5.1.1), an enzyme hydrolyzing asparagine to aspartic acid and ammonia, is included in most treatment schedules for acute lymphoblastic leukemia (ALL), the most common form of leukemia in children. The leukemic blast cells that cause ALL do not express sufficient amounts of asparagine synthase and therefore require exogenous asparagine for growth. When asparagine is depleted in the blood by asparaginase administration, the protein synthesis of the blasts is compromised, leading to the impairment of cellular functions and eventually to apoptosis (Sobin and Kidd, 1965; Becker and Broome, 1967, 1969; Cooney and Handschuhmacher, 1970). It is assumed, but not yet established, that the enzyme is eliminated via the reticuloendothelial system (Broome, 1968a,b; Hall, 1970).

The efficiency of asparaginase treatment has been proven in a large number of clinical trials. For instance, two controlled randomized studies showed that patients treated with asparaginase in addition to a standard chemotherapeutic regimen had a much superior disease-free survival compared to standard chemotherapy alone

(Sallan et al., 1983; Desai et al., 1997). This was confirmed unintentionally in a recent clinical trial of 700 patients, in which the different pharmacokinetics of the asparaginases from *E. coli* (EcA) and *Erwinia chrysanthemi* (ErA) had been neglected, which led to inferior results for patients treated with the less active *Erwinia* enzyme (Otten et al., 1996; Pacquement et al., 1997; Duval et al., 2002).

At present, asparaginase II from *E. coli* is the only asparaginase commercially available for ALL therapy. It is applied either in its native state or in pegylated form, i.e., conjugated with polyethylene glycol (PEG) for prolongation of its half-life and probably for reduction of immunogenicity. As EcA is a bacterial protein, a major limitation of asparaginase treatment involves immunological reactions, which range from minor processes at the injection site to sometimes fatal systemic reactions such as anaphylaxis (Müller et al., 2001). In a variety of clinical investigations it was found that the incidence of early-type immunological reactions to EcA administration varied between less than 5% and 75%. As anticipated, the probability of the occurrence of hypersensitivity reactions increases with the number of doses within one cycle and has its peak at the re-start of asparaginase administration after asparaginase-free intervals (Dellinger and Miale, 1976; Evans et al., 1982; Müller et al., 2000, 2001).

In addition to allergic reactions, another frequent complication of asparaginase therapy is a rapid decline in asparaginase activity in the blood without any clinical symptoms, the so-called 'silent inactivation' (Asselin et al., 1993; Kurtzberg et al., 1993; Ahlke et al., 1997; Müller et al., 2000). As this phenomenon can reduce the half-life of pegylated EcA *in vivo* from the usual 1–2 weeks to less than 1 day, it will abolish the therapeutic effect without being noticed. To avoid this, a 'drug monitoring' procedure is used, in which blood samples from patients are analyzed for asparaginase activity for approximately 2 weeks after enzyme administration. Although it has been claimed that pegylated asparaginases are less immunogenic, clinical investigations showed that silent inactivation of such preparations also occurs in a considerable number of patients (Park et al., 1981; Yoshimoto et al., 1986; Müller et al., 2000).

It is reasonable to assume that both hypersensitivity reactions and silent inactivation are mediated by anti-asparaginase antibodies formed after the first asparaginase administration (the so-called induction treatment). The detection of such antibodies in the blood of patients could be a way of predicting possible complications of asparaginase treatment. Unfortunately, enzyme-linked immunosorbent assays (ELISAs) with the native enzyme as antigen failed to show a clear correlation between antibody levels and the clinical picture (Woo et al., 1998; Wang et al., 2003). Other researchers, using a similar ELISA technique, found a correlation between high anti-

body titers and low serum activity in patients treated with native asparaginase, but not in patients treated with the identical pegylated enzyme, although in this group a rapid decline in serum activity was also observed in some patients (Avramis et al., 2002). Based on these observations, several hundreds of volunteers who had never been exposed to asparaginase were tested for anti-asparaginase antibodies. The results were rather surprising, since significant (in some cases rather high) antibody titers were measured in the serum of nearly all of the volunteers.

To develop more reliable methods to predict hypersensitivity reactions and silent inactivation, we started a project with the aim of developing an epitope-specific assay of anti-asparaginase antibodies. To this end, we first focused on the identification of major B-cell epitopes on the surface of *E. coli* asparaginase II, which is at present the only enzyme of therapeutic importance. Such information should facilitate the development of a more specific assay whereby synthetic peptides can be used to detect clinically relevant anti-EcA antibodies. Two independent techniques were used for epitope mapping. The first one involved screening against anti-EcA antibodies of a phage-displayed random peptide library ('phage display'), while the second one relied on the SPOTs technique, in which overlapping synthetic decapeptides spanning the entire EcA sequence, synthesized as spots on a cellulose membrane, served as antigens. Here we show that most peptides identified by either method align

to epitopes on the surface of the EcA molecule. The position of several 'strong' epitopes relative to the enzyme's active site offers an explanation as to why silent inactivation is such a common pitfall in asparaginase treatment.

Results

Antigenic peptides identified by phage display

As detailed in the materials and methods section, a commercial library of random heptapeptides fused to the pIII protein of phage M13 was screened against a polyclonal rabbit anti-EcA serum. A total of 59 single colonies were picked at random from the eluate of the third panning round for DNA sequencing. In 48 cases (81%), the phage-displayed peptides could be matched with partial sequences of EcA in a satisfactory way. The criteria applied were: (i) at least three exact matches; (ii) at least six sequence similarities (i.e., polar or non-polar residues in the same position); and (iii) no gaps allowed. In Figure 1, the corresponding peptide sequences are aligned with the EcA sequence. In each case, identities are highlighted by bold letters. In addition, the solvent accessibility of each residue in the EcA tetramer was estimated from PDB entry 3ECA using DSSP (Kabsch and Sander, 1983). Residues with calculated accessibility (ACC) values of



Figure 1 Alignment of the *E. coli* asparaginase amino acid sequence with antigenic peptides identified by the SPOTs technique (above) or phage display (below). For further details see the text.

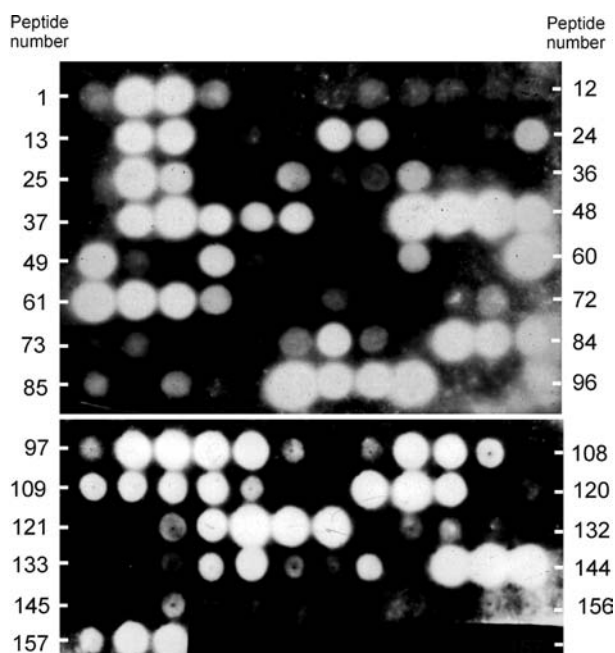


Figure 2 SPOTs membranes containing 159 overlapping decapeptides spanning the *E. coli* asparaginase sequence after antibody probing with chemiluminescent detection.

50–100 Å² are single underlined and those with ACC > 100 Å² are double underlined.

Interestingly, approximately two-thirds of all matching peptides were identical with or closely related to the EcA sequence SVFDTLA (residues 252–258), indicating that the polyclonal antiserum used contained especially tight-binding antibodies directed against this particular sequence. Five peptides matched residues 53–59 (VVNIGSQ), three peptides were related to residues 201–207 (TPFDVSK), and two to residues 129–135 (LYAAINL). All others aligned to different sites.

Peptides identified by the SPOTs approach

The SPOTs technique allows the combinatorial synthesis of peptides on a cellulose membrane support. In the present study, we synthesized 159 overlapping decapeptides with an offset of two residues that span the entire EcA sequence. The resulting membranes were screened with the same polyclonal antiserum used for panning in the phage display experiments. Typical results of the SPOTs screening are illustrated by Figure 2, which shows two membranes containing all 159 peptides after chemiluminescent visualization of bound antibodies. In this particular experiment, many spots are saturated due to the high sensitivity of the stain, so that quantitative information is difficult to derive from these data. Therefore, in consecutive series of reactive peptides those yielding the most intense signal were identified from membranes stained by the less sensitive non-chemiluminescent method (data not shown).

The positions of the most reactive peptides in the primary structure of EcA are shown in Figure 1, which allows comparison with the results of phage display. Clearly, the majority of the peptides giving strong signals on SPOTs membranes map to regions that were also

identified by phage display. Exceptions are the peptides numbered 39, 46, 82, 142–144, and 158–159.

Discussion

B-Cell epitopes are defined as groups of amino acids that are clustered together on the surface of a protein and determine its antigenicity, as they are recognized by antibodies and B-cell receptors. Whether peptides identified by scanning of combinatorial libraries always correspond to native epitopes of the target protein is still a matter of debate. Linear peptides that are recognized by antibodies frequently correspond to flexible loops on the protein surface. Why they cross-react with the corresponding sequences embedded in the tertiary structure of the protein is still not clear (Craig et al., 1998). It is generally accepted, however, that epitopes identified by peptide methods represent only a small fraction of the total epitopes present, most of which are discontinuous, i.e., composed of several parts of the peptide chain grouped together on the surface. Thus, antibodies raised against the native protein will recognize short linear peptides only if they contain an important part of a dominant native epitope (Van Regenmortel, 1996).

Our present data suggest that at least some of the linear epitopes identified in EcA meet this criterion. First, the same peptides were recognized regardless of whether they were immobilized on a SPOTs membrane via their C-termini or fused to a phage surface protein. Second, as suggested by the accessibility parameters included in Figure 1, most of the peptides identified correspond to partial sequences located at or close to the protein surface. It should be noted, however, that the accessibility data were calculated from the structure of the native tetramer. It is now well established that dilute asparaginase solutions also contain significant amounts of dimers in which additional residues are accessible (Verma and Röhm, unpublished data). SPOTs peptide 82, for instance, is largely inaccessible in the tetramer but is solvent-exposed in dimers. Conformational changes that accompany substrate binding to EcA could also markedly influence the accessibility of residues. During catalysis the active sites of the bacterial asparaginases are shielded from the solvent by mobile peptide loops that, in EcA, are formed by residues 14–26 of each subunit. In the absence of substrate, these 'lids' are open and conformationally highly flexible. It is only the binding of asparagine or the competitive inhibitor aspartate that induces loop closure, thereby markedly stabilizing the protein (Aung et al., 2000). Some epitopes may therefore only exist in the free enzyme or in the enzyme substrate complex.

In 1994 Moola and co-workers published the results of a hexapeptide scan to detect epitopes in *Erwinia chrysanthemi* asparaginase (ErA), a bacterial asparaginase that was in clinical use until recently (Moola et al., 1994). In that study, the only antigenic peptide consistently recognized by several antisera against the *Erwinia* enzyme contained a sequence that corresponds to residues 285–289 (in ErA numbering). The dominant *E. coli* epitope identified in the present study (SVFDTLA) did not

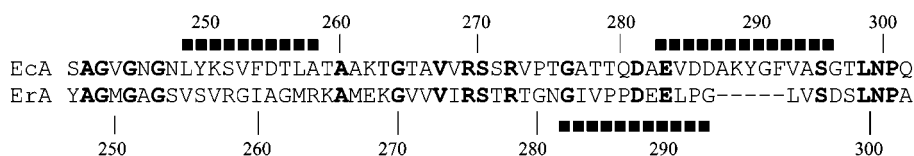


Figure 3 Comparison of epitopes in the C-terminal domains of *E. coli* (EcA) and *Erwinia chrysanthemi* (ErA) asparaginase. For further details see text.

react with antibodies raised against ErA. This is not surprising, as the respective regions in the two enzymes show only low sequence similarity (see Figure 3). The tandem of proline residues contained in the ErA epitope PPDEE (P-285 and P-286), which were shown to be essential for antibody binding, is replaced by other residues in EcA. Moreover, the *E. coli* enzyme contains an extra loop in that region that is also highly antigenic. The immunodominant EcA sequence 249–259, on the other hand, is not conserved in the *Erwinia* enzyme. These findings are consistent with previous observations that EcA and ErA are only weakly cross-reactive (Hrushesky et al., 1976). It is for this reason that ErA has frequently been substituted for the *E. coli* enzyme when allergic reactions against the latter developed in patients during asparaginase treatment.

When the antigenic peptides identified in the present study are mapped to the surface of the EcA tetramer, spatial relationships become apparent that may explain the phenomenon of silent inactivation encountered in ALL therapy. While the EcA monomers are identical, the native EcA tetramer is actually a dimer of dimers, in which subunits A and C or B and D, respectively, form 'close pairs' (Swain et al., 1993). The four active sites are each made up from the N-terminal domain of a given subunit and the C-terminal domain of the other member of the respective close pair. This is illustrated by Figure 4A, in which the A/C pair is depicted in shades of blue and the B/D pair in red. Figure 4B shows in more detail the active site of subunit D, recognizable by the bound aspartate (Asp). The mobile loop (in its closed position) is highlighted in blue. Three dominant epitopes surround

the active site, i.e., residues 53–58 of subunit D (corresponding to SPOTs peptide 26, light red), amino acids 253–257 of subunit B (dark red, peptide 126), and residues 283–289, also from subunit B (pink, peptide 143). The side chains of the most exposed residues in these sequences (N55, D255, and K288) are shown as cpk models. In the native EcA tetramer, sequences 53–58 and 283–289 form loops on the protein surface. Note that two of these residues (S58 and E283) are in immediate contact with bound substrates or inhibitors. Residues 253–257 are constituents of a long amphipathic helix, in which the side chains of K251, D255, T259, and K262 are exposed on the protein surface.

In Figure 4C the respective regions are mapped to the solvent-accessible surface of the B/D-dimer. Clearly, all of these epitopes are so close to the mobile loop that antibody binding to any of them is likely to block loop movement. As discussed above, this would abolish catalysis, which crucially depends on the opening and closing of the loop. We therefore suggest that antibodies directed against some or all of the epitopes shown in Figure 4 are responsible for the silent inactivation of EcA observed in ALL patients. Furthermore, from Figure 4C it appears that the epitopes on subunit B are contiguous. Thus, they could be parts of one larger discontinuous (conformative) epitope.

Of course, the relevance of the epitopes detected in the present study will have to be confirmed and quantitatively assessed by independent techniques. We have already started mutagenesis experiments with the aim of identifying residues that are crucial for antibody binding to the individual sequences in the native protein. Fur-

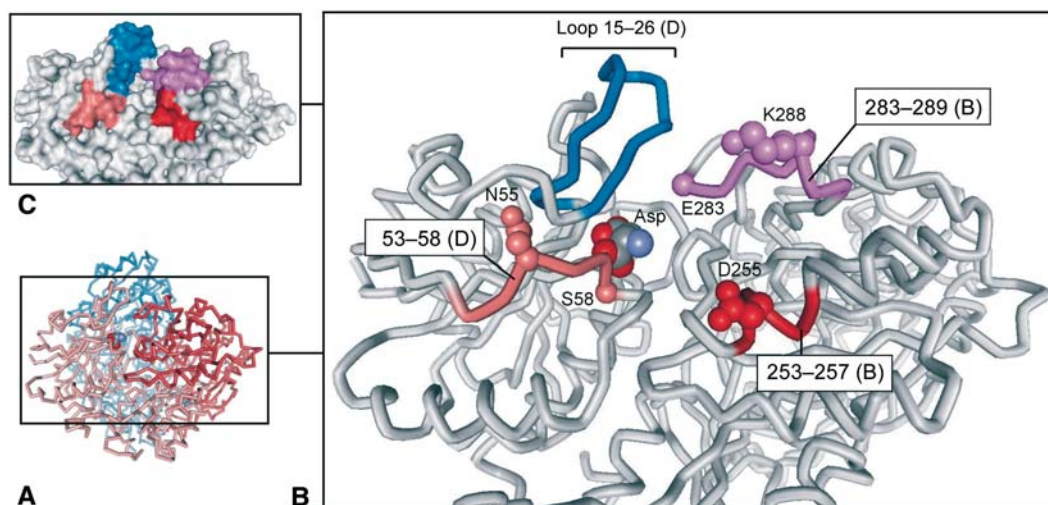


Figure 4 Position of dominant epitopes close to the active site of EcA. For further details see the text.

thermore, to identify extended discontinuous epitopes, we will screen larger protein fragments against the available antibodies (Reineke et al., 1998). Finally, and most importantly in the clinical context, we will have to define the epitopes that are associated with the already mentioned serious side effects of asparaginase treatment. This will be performed by screening our peptide libraries against antisera derived from healthy persons, as well as from asparaginase-treated patients with or without hypersensitivity reactions or silent inactivation. If such correlations can be established, the data would provide a reliable basis for simplified and more efficient monitoring of asparaginase therapy.

Materials and methods

Antibodies

For phage display, as well as SPOTs screening, the immunoglobulin G (IgG) fraction of a commercially available rabbit anti-*E. coli*-asparaginase antiserum was used (Rockland Inc., Gilbertsville, USA; code 200-4171, lots #1097 and #11587).

Phage display

For epitope screening the Ph.D.-7 Phage Display Peptide Library Kit from New England Biolabs (Beverly, USA) was used. This kit is based on a combinatorial library of random peptide 7-mers fused to a minor coat protein (pIII) of the M13 phage. The library has a complexity of approximately 2.8×10^9 independent clones, representing the $20^7 = 1.28 \times 10^9$ possible 7-residue sequences. Because M13 is a male-specific coliphage, for M13 propagation the *E. coli* F⁺ strain ER2738 supplied with the kit was used. The F-factor of ER2738 contains a mini-transposon that confers tetracycline resistance, and thus cells harboring the F-factor can be selected by tetracycline-containing media. Infectivity is mediated by binding of the M13 coat protein pIII to the F-pilus of the recipient bacterium. Discrimination on culture plates between library phage plaques and plaques caused by environmental filamentous phage is permitted by the implemented *lacZα* gene, as library phage plaques appear blue when plated on media containing Xgal and isopropyl-β-D-thiogalactoside (IPTG). Because the number of plaques increases linearly with the amount of phage only when cells are in excess, phage stocks of the panning rounds were titrated by diluting prior to infection. For estimation of phage titer in plaque forming units (pfu) per 10 μl, plates containing approximately 10² plaques were used. Panning rounds were then performed with an input volume corresponding to approximately 2×10^{11} pfu.

For binding of 7-mers presented on pIII, 150 μg of the IgG fraction from anti-*E. coli* asparaginase antibodies in 1.5 ml of 0.1 M NaHCO₃ was immobilized on NUNC Maxisorp 4-44202 tubes. This was carried out in triplicate, i.e., three identical investigations were performed in parallel, and therefore all the steps described below were carried out in three independent experiments. After pouring off the coating solution, the tubes were incubated with blocking buffer followed by washing steps. Binding of phage was then enabled for 30 min at room temperature, discarding non-binding phages thereafter. After several washing steps, bound phages were eluted and amplified in ER2738 the following day. The cultures containing the phages amplified in ER2738 were centrifuged and the upper 80% of the supernatant was mixed with 1/6 volume of PEG/NaCl for phage precipitation overnight. On the following day, after centrifugation the pellet was resuspended in buffer and spun again to pellet residual cells. The supernatant was supplemented again with 1/6 volume

of PEG/NaCl for re-precipitation. After centrifugation the pellet was suspended in Tris-buffered saline with 0.02% NaN₃ and centrifuged to pellet any remaining insoluble matter. The supernatant containing the amplified eluate was then used for the following panning round, which after titration was performed with an input volume corresponding to approximately 2×10^{11} pfu. The unamplified third-round elute was titrated then as described, and plaques from plates after amplification in ER2738 with approximately 100 single colonies were used for sequencing. As three independent experiments were performed in parallel, 10, 30, and 25 single colonies were picked for sequencing from the different investigations. Overall, DNA from 59 single colonies could be used for sequencing of the single-stranded phage DNA, which was performed by MWG Biotech (Ebersberg, Germany). Alignment of selected heptapeptide-pIII fusions was performed against the sequence of the *E. coli* asparaginase (EC 3.5.1.1), which is the active component of the drug Asparaginase medac™, the asparaginase most frequently used in the treatment of acute lymphoblastic leukemia in Germany.

Peptide synthesis by SPOTs

For epitope mapping at the protein level, the SPOTs kit from Sigma Genosys Biotechnologies (The Woodlands, TX, USA) was applied. The method offers the possibility to synthesize up to 96 peptides simultaneously as small circular spots on a derivatized cellulose sheet and subsequently assay them for immunological reactivity. Synthesis is performed by Fmoc-L-amino acid esters in different pipetting cycles according to the length of the peptides. For epitope screening of the asparaginase protein, decapeptides with an offset of two amino acids spanning the whole amino acid sequence of EcA were synthesized on two membranes (159 spots).

For the coupling reaction, the Fmoc-amino acid esters had to be dissolved in 1-methyl-2-pyrrolidinone (NMP), whereas the washing steps were performed with *N,N*-dimethylformamide (DMF). Since both solvents can decompose to give free amines, which may interfere with peptide synthesis, free amines were removed with 4-Å molecular sieves prior to use. A check for free amines was performed with a 1% bromophenol blue solution, which turns from yellow to green/blue in the presence of free amines. According to the manufacturer's instructions, the coupling step for each amino acid was repeated a second time before the next amino acid was added. After each synthesis cycle, the membrane was acetylated after washing with DMF to block any free amino group. Activation of the N-terminus of the last added amino acid was achieved using a 20% piperidine solution prior to the next synthesis step. After binding of the final amino acid, trifluoroacetic acid was applied for side-chain deprotection. Probing of the peptide spots was performed according to the manufacturer's instructions.

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References

- Ahlke, E., Nowak-Göttl, U., Schulze-Westhoff, P., Werber, G., and Börste, H. (1997). Dose reduction of asparaginase under pharmacokinetic and pharmacodynamic control during

- induction therapy in children with acute lymphoblastic leukaemia. *Br. J. Haematol.* 96, 675–681.
- Asselin, B., Gelber, R., and Sallan, S. (1993). Relative toxicity of *E. coli* L-asparaginase (ASP) and PEG-asparaginase (PEG) in newly diagnosed childhood acute lymphoblastic leukemia (ALL). *Med. Pediatr. Oncol.* 21, 556.
- Aung, H.P., Bocola, M., and Röhm, K.H. (2000). Dynamics of a mobile loop at the active site of *E. coli* asparaginase. *Biochim. Biophys. Acta* 1481, 349–59.
- Avramis, V.I., Sencer, S., Periclou, A.P., Sather, H., Bostrom, C., Cohen, L.J., Ettinger, A.G., Ettinger, L.J., Franklin, J., Gaynon, P.S., et al. (2002). A randomized comparison of native *Escherichia coli* asparaginase and polyethylene glycol-conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. *Blood* 99, 1986–1994.
- Becker, F.F. and Broome, J.D. (1967). L-Asparaginase: inhibition of early mitosis in regenerating rat liver. *Science* 156, 1602–1603.
- Becker, F.F. and Broome, J.D. (1969). L-Asparaginase: inhibition of endogenous RNA polymerase activity in regenerating liver. *Arch. Biochem. Biophys.* 130, 332–336.
- Broome, J.D. (1968a). Studies on the mechanism of tumor inhibition by L-asparaginase. *J. Exp. Med.* 127, 1055–1072.
- Broome, J.D. (1968b). Factors which may influence the effectiveness of L-asparaginase as tumor inhibitors. *Br. J. Cancer* 22, 595.
- Cooney, D. and Handschumacher, R. (1970). L-Asparaginase and L-asparagine metabolism. *Annu. Rev. Pharmacol.* 10, 421–440.
- Craig, L., Sanschagrin, P.C., Rozek, A., Lackie, S., Kuhn, L.A., and Scott, J.K. (1998). The role of structure in antibody cross-reactivity between peptides and folded proteins. *J. Mol. Biol.* 281, 183–201.
- Dellinger, C.T. and Miale, T.D. (1976). Comparison of anaphylactic reactions to asparaginase derived from *Escherichia coli* and from *Erwinia* cultures. *Cancer* 38, 1843–1846.
- Desai, N., Amylon, M., Schwenn, M.R., Laver, J., and Murphy, L. (1997). Outcome of advanced stage (III–IV) lymphoblastic non-Hodgkin's lymphoma – a Pediatric Oncology Group (POG) study. *Med. Pediatr. Oncol.* 29, 356 (Abstract O-140).
- Duval, M., Suci, S., Ferster, A., Rialland, X., Nelken, B., Lutz, P., Benoit, Y., Robert, A., Manel, A.M., Vilmer, E., et al. (2002). Comparison of *Escherichia coli*-asparaginase with *Erwinia*-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer – Children's Leukemia Group phase 3 trial. *Blood* 99, 2734–2739.
- Evans, W.E., Tsiatis, A., Rivera, G., Murphy, S.B., Dahl, G.V., Denison, M., Crom, W.R., Barker, L.F., and Mauer, A.M. (1982). Anaphylactoid reactions to *Escherichia coli* and *Erwinia* asparaginase in children with leukemia and lymphoma. *Cancer* 49, 1378–1383.
- Hall, J.G. (1970). The partitioning of L-asparaginase between blood and lymph. *Recent Results Cancer Res.* 33, 75–80.
- Hrushesky, W.J., Slavik, M., Penta, J., and Muggia, F. (1976). The 'other' asparaginase. *Med. Pediatr. Oncol.* 2, 441–442.
- Kabsch, W. and Sander, C. (1983). Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers* 22, 2577–2637.
- Kurtzberg, J., Asselin, B., Poplack, D., Grebanier, A., Chen, R., Franklin, A., Scudiero, D., and Fisherman, J. (1993). Antibodies to asparaginase alter pharmacokinetics and decrease enzyme activity in patients an asparaginase therapy. *Proc. Am. Assoc. Cancer Res.* 34, 304.
- Moola, Z.B., Scawn, M.D., Atkinson, T., and Nicholls, D.J. (1994). *Erwinia chrysanthemi* L-asparaginase: epitope mapping and production of antigenically modified enzymes. *Biochem. J.* 302, 921–927.
- Müller, H.J., Löning, L., Horn, A., Schwabe, D., Gunkel, M., Schrappe, M., von Schütz, V., Henze, G., da Palma, J.C., Ritter, J., et al. (2000). Pegylated asparaginase (Oncaspar) in children with ALL: drug monitoring in reinduction according to the ALL/NHL-BFM 95 protocols. *Br. J. Haematol.* 110, 379–384.
- Müller, H.J., Beier, R., Löning, L., Blüters-Sawatzki, R., Dörffel, W., Maass, E., Müller-Wehrich, S., Scheel-Walter, H.G., Scherer, F., Stahnke, K., et al. (2001). Pharmacokinetics of native *Escherichia coli* asparaginase (Asparaginase Medac) and hypersensitivity reactions in ALL-BFM 95 reinduction treatment. *Br. J. Haematol.* 114, 794–799.
- Otten, J., Suci, S., Lutz, P., Benoit, Y., Robert, A., Thyss, A., Plouvier, E., Ferster, A., Mechinaud, F., Mazingue, F., et al. (1996). The importance of L-asparaginase (A'ASE) in the treatment of acute lymphoblastic leukemia (ALL) in children: results of the EORTC 58881 randomized phase III trial showing greater efficiency of *Escherichia coli* (*E. coli*) as compared to *Erwinia* (ERW) A'ASE. *Blood* 88 (Suppl. 1/I), 669a.
- Pacquement, H., Philippe, N., Mechinaud, F., Robert, A., Vilmer, E., Francotte, N., and Millot, F. (1997). Importance of L-asparaginase (Asp), detrimental effects of additional cytosine-arabioside (Ara-C) and of iv 6-mercaptopurine (6-MP) in the treatment of lymphoblastic non-Hodgkin lymphoma (LB-NHL): results of the EORTC 58881 randomized trial. In: *SIOP XXIX, Abstract P-160*, p. 429.
- Park, Y.K., Abuchowski, A., Davis, S., and Davis, F. (1981). Pharmacology of *Escherichia coli*-L-asparaginase polyethylene glycol adduct. *Anticancer Res.* 1, 373–376.
- Reineke, U., Sabat, R., Volk, H.D., and Schneider-Mergener, J. (1998). Mapping of the interleukin-10/interleukin-10 receptor combining site. *Protein Sci.* 7, 951–960.
- Sallan, E., Hitchcock-Bryan, S., Gelber, R., Cassady, J., Frei, E., and Nathan, G. (1983). Influence of intensive asparaginase in the treatment of childhood non-T-cell acute lymphoblastic leukemia. *Cancer Res.* 43, 5601–5607.
- Sobin, L.H. and Kidd, J.G. (1965). A metabolic difference between two lines of lymphoma 6C3HED cells in relation to asparaginase. *Proc. Soc. Exp. Biol. Med.* 119, 325–327.
- Swain, A.L., Jaskólski, M., Housset, D., Rao, J.K.M., and Wlodawer, A. (1993). Crystal structure of *Escherichia coli* L-asparaginase, an enzyme used in cancer therapy. *Proc. Natl. Acad. Sci. USA* 90, 1474–1478.
- Van Regenmortel, M.H.V. (1996). Mapping epitope structure and activity: from one-dimensional prediction to four-dimensional description of antigenic specificity. *Methods* 9, 465–472.
- Wang, B., Relling, M.V., Storm, M.C., Woo, M.H., Ribeiro, R., Pui, C.H., and Hak, L.J. (2003). Evaluation of immunologic cross-reaction of anti-asparaginase antibodies in acute lymphoblastic leukemia (ALL) and lymphoma patients. *Leukemia* 17, 1583–1588.
- Woo, M.H., Hak, L.J., Storm, M.C., Evans, W.E., Sandlund, J.T., Rivera, G.K., Wang, B., Pui, C.H., and Relling, M.V. (1998). Anti-asparaginase antibodies following *E. coli* asparaginase therapy in pediatric acute lymphoblastic leukemia. *Leukemia* 12, 1527–1533.
- Yoshimoto, T., Nishimura, H., Saito, Y., Sakurai, K., Kasimaki, Y., Wada, H., Sako, M., Tsujino, G., and Inada, Y. (1986). Characterization of polyethylene glycol-modified L-asparaginase from *Escherichia coli* and its application to therapy of leukemia. *Jpn. J. Cancer Res.* 77, 1264–1270.

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