Research paper

In vitro pyrogen test for toxic or immunomodulatory drugs

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Abstract

Pyrogenic contaminations of some classes of injectable drugs, e.g. toxic or immunomodulatory as well as false-positive drugs, represent a major risk which cannot yet be excluded due to the limitations of current tests. Here we describe a modification of the In vitro Pyrogen Test termed AWIPT (Adsorb, Wash, In vitro Pyrogen Test), which addresses this problem by introducing a pre-incubation step in which pyrogenic contaminations in the test sample are adsorbed to albumin-coated beads. After rinsing, the beads are incubated with human whole blood and the release of the endogenous pyrogen interleukin-1 β is measured as a marker of pyrogenic activity. Intentional contaminations with lipopolysaccharide were retrieved from the chemotherapeutic agents paclitaxel, cisplatin and liposomal daunorubicin, the antibiotic gentamicin, the antifungal agent liposomal amphotericin B, and the corticosteroid prednisolone at lower dilutions than in the standard in vitro pyrogen test. This represents a promising new approach for the detection of pyrogenic contamination in drugs or in drugs containing interfering additives and should lead to improved safety levels.

Keywords: Whole blood test; Lipopolysaccharide; Pyrogen testing; Interleukin-1 β

1. Introduction

Pyrogens are fever-inducing substances usually derived from microorganisms. While lipopolysaccharide (LPS) from the outer membrane of Gram-negative

bacteria remains the prototypic pyrogen, substances such as lipoteichoic acid (LTA) and peptidoglycan from Gram-positive bacteria and others are increasingly recognized as potent immune stimuli (Morath et al., 2001; Schindler et al., 2003; Artis et al., 2005). The presence of pyrogens in the body initiates secretion of pro-inflammatory cytokines by monocytes. When high concentrations of pyrogens enter the blood stream they cause fever, septic shock or even death (Cohen, 2000; Hartung et al., 2001). Therefore, pyrogen testing of parenteral drugs was introduced into pharmacopoeias worldwide in the 1940s.

The rabbit pyrogen test measures the change in body temperature after injection of the test substance. Major

Abbreviations: AWIPT, Adsorb, Wash, In vitro Pyrogen Test; BET, bacterial endotoxin test; EU, endotoxin unit; IL-1 β , interleukin-1 β ; IPT, in vitro pyrogen test; LAL, Limulus amoebocyte lysate assay; LPS, lipopolysaccharide; LTA, lipoteichoic acid.

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drawbacks are (i) higher threshold concentrations compared to humans resulting in a lower sensitivity (Schindler et al., 2003), (ii) variations in the response depending on sex, age and strain (El-Khalik and Benoliel, 1982; Deeter et al., 1989; Hull et al., 1993); (iii) differences between the reactions of rabbits and humans towards different pyrogen classes (Schindler et al., 2003), (iv) the rabbit pyrogen test is not suitable for sera, radiopharmaceuticals, chemotherapeutics, analgesics, cytokines, immunosuppressive agents and others (Hartung et al., 2001).

The *Limulus* amoebocyte lysate assay (LAL, also known as bacterial endotoxin test, BET), established since the 1970s, is based on the coagulation cascade of the hemolymph of an arachnid, the horseshoe crab, which is induced by LPS in concentrations as low as 3 pg/ml. Kinetic and chromogenic LAL test variants permit the quantification of LPS. However, the LAL has several disadvantages, e.g. (i) it only detects LPS, thus overlooking other clinically relevant pyrogens (van Noordwijk and de Jong, 1977; Hartung et al., 2001), (ii) it gives false positive results with glucan-like structures and many herbal medicines, (iii) it does not reflect the potency of the LPS in humans (Dehus et al., 2006) and (iv) various substances interfere with LPS detection in the LAL (van Noordwijk and de Jong, 1977; Hartung et al., 2001). As an alternative the *In vitro* Pyrogen Test (IPT) models the human endogenous fever response. It measures the release of the fever-mediator interleukin-1 β (IL-1 β) in human whole blood upon incubation with the sample substance (Hartung and Wendel, 1995). This test has been validated as a full alternative to the rabbit pyrogen assay in a collaborative EU-funded study (Schindler et al., 2001; Hoffmann et al., 2005).

The whole blood test recognizes all known pyrogens that are relevant to humans and reflects their relative potency (Fennrich et al., 1999). However, some therapeutic formulations, i.e. either the drug itself or additives, will interfere with this test, e.g. by restricting the response of white blood cells due to toxic or immunomodulatory effects. Indeed, the very same substances pose difficulties in the rabbit test. In the present study, we have addressed such problems using macroporous acrylic beads decorated with immobilized human serum albumin. The material was originally developed as an extracorporeal endotoxin adsorbing matrix to treat endotoxemia and sepsis (Zimmermann et al., 1999; Ullrich et al., 2001; Staubach et al., 2003). Albumin is a universal carrier of lipophilic substances such as fatty acids, bilirubin and hormones in plasma (Bhattacharya et al., 2000; Petitpas et al., 2001) and also

binds LPS, largely via hydrophobic interactions in a molar ratio of up to 10:1 (Jurgens et al., 2002).

We show here that a modification of the IPT allows LPS spikes to be detected in complex solutions with a lower detection limit than with the standard IPT by adsorbing them to albumin-coated beads and then washing away the interfering substances before incubation of the beads with blood and detection of cytokine release.

2. Materials and methods

2.1. Pyrogens

Endotoxin from *Escherichia coli* O-113 (EC-6, a kind gift from Dr. S. Poole, National Institute of Biological Standard and Controls, Herts, UK) was used. 100 pg of this LPS are defined as 1 EU (Poole and Mussett, 1989). LTA from *Staphylococcus aureus* was isolated and prepared in-house as described previously (Morath et al., 2001). Spike concentrations described in the text and figures always refer to the concentration in the sample solution.

2.2. Drug samples

The drug samples were pharmaceutical preparations with the following recommended maximum daily allowances: paclitaxel (Taxol, Bristol-Myers Squibb), 4.25 mg/kg; cisplatin (Cisplatin, HEXAL), 3 mg/kg; gentamicin (Gentamicin, HEXAL), 14 mg/kg; prednisolone (Prednisolut, Jenapharm), 6 mg/kg; liposomal amphotericin B (AmBisone, NeXstar), 3 mg/kg; and liposomal daunorubicin (DaunoXome, NeXstar), 1 mg/kg, according to the package insert, assuming a body weight of 70 kg and 1.7 m² body surface.

The drug samples were diluted in saline, then spiked with endotoxin and incubated at room temperature for 2 h before commencement of the AWIPT (see below). This pre-incubation period allows for possible binding of endotoxin to drug components.

2.3. *In vitro* pyrogen test (IPT)

Heparinized blood was drawn from healthy donors by venous puncture. Differential blood cell counts were routinely performed to rule out acute infections (Pentra 60, ABX Diagnostics). 100 μ l test sample were diluted with 1 ml 0.9% saline and incubated with 100 μ l blood overnight at 37 °C and 5% CO₂ in a 1.5 ml polypropylene reaction vial. After incubation, samples were resuspended and spun down (2 min at

13,000×g) and supernatants were stored at -80°C until cytokine measurements could be performed. Interleukin-1 β was quantified using an in-house sandwich ELISA based on a matched antibody pair (R&D Systems). Binding of biotinylated antibody was quantified using streptavidin-peroxidase (Biosource) and the substrate 3,3',5,5'-tetramethylbenzidine (Sigma-Aldrich). Recombinant IL-1 β was a generous gift from Dr. S. Poole (NIBSC).

2.4. Preparation of albumin beads

Macroporous acrylic beads (Eupergit, Degussa) decorated with human serum albumin, fraction V (low endotoxin) (Serva, Heidelberg, Germany) via covalent carboxyl linkage as described previously (Zimmermann et al., 1999; Zimmermann, 2000) were provided by Fresenius Hemocare (St. Wendel, Germany) in vacuum sealed sterile cartridges. Approximately 2.5 g were transferred into 50 ml polypropylene tubes (Greiner) and rinsed with 50 ml 0.9% saline (Berlin Chemie) to remove residual unbound albumin. After sedimentation of the beads, the supernatant was removed and the beads were rinsed with 50 ml priming solution (4 mM K $^{+}$, 1.75 mM Ca $^{2+}$, 0.5 mM Mg $^{2+}$, 106.5 mM Cl $^{-}$, 134 mM Na $^{+}$, 36 mM HCO $_{3}^{-}$, 5.7 mM citrate, 6.2 mM dextrose, pH 7.2, provided by Fresenius Hemocare). 40 ml of supernatant were removed and 2% v/v DMSO (Cryosure, WAK Chemie) were added to reduce the buoyancy of the beads.

2.5. AWIPT (adsorption, washing and in-vitro pyrogen test)

Adsorption: 100 μl bead suspension, equivalent to 53 mg beads and approximately 0.8 mg albumin (Zimmermann et al., 1999; Zimmermann, 2000), were transferred to a 2 ml reaction tube (Eppendorf). 100 μl sample or LPS spike were added to the beads, and the rack with the closed tubes was incubated horizontally overnight at 4°C to allow efficient adsorption.

Washing: 1 ml priming solution supplemented with 0.025% Tween-20 (Sigma, cell culture grade) was added to the beads and removed as completely as possible using a suction pump after sedimentation (approximately 2 min). This procedure was repeated three times with priming solution without Tween-20.

IPT: The whole blood incubation protocol (see above) was refined as follows: to each bead-containing tube, 250 μl saline (Berlin Chemie) and 100 μl heparinized blood of a healthy donor were added, and incubated overnight at 37°C in the presence of 5% CO $_2$.

2.6. Data analysis

According to the standard operating procedure of the IPT, the IL-1 β secretion in response to the LPS spike must be greater than the mean of the control samples plus three times their standard deviation for the test to be valid. A sample was deemed interfering with pyrogen detection if the sample spiked with LPS induced IL-1 β release that was less than 50% or more than 200% of IL-1 β released in response to the same spike concentration in the absence of sample.

3. Results

3.1. Detection of endotoxin with AWIPT

The protocol was optimized with regard to the number of beads, saline volume, blood volume and washing steps with the aim of reaching a detection limit of at least 50 pg/ml LPS, which corresponds to the fever threshold of the most sensitive rabbit strains. A representative example of an endotoxin dilution curve in saline is shown in Fig. 1. Physiological saline was spiked with a concentration series of LPS (25 pg/ml LPS to 1600 pg/ml LPS) and underwent the AWIPT procedure. Concentration dependent LPS-induced IL-1 β release was observed. The indicated cut-off (38 pg/ml IL-1 β), defined as the mean of the negative control plus its threefold standard deviation, corresponds to the border value above which a single value is considered positive with $p=0.99$. The amounts of IL-1 β induced by 25 pg/ml endotoxin were already significantly different from the negative controls (blank). In Fig. 1A, the sigmoidal regression of the curve ($r^2=0.86$) demonstrated saturation effects of AWIPT at high concentrations. As shown in Fig. 1B, linear approximation gave a similar fit ($r^2=0.85$) over a limited concentration range (0–200 pg/ml).

3.2. Comparison of the LPS binding capacity of unmodified and albumin-coupled beads

Native and albumin-coupled beads were incubated with different concentrations of LPS for 2 h and then employed in the AWIPT procedure. Fig. 2 shows that a concentration dependent response of IL-1 β secretion only occurred in the presence of the albumin-coupled beads. This suggests that the coupled albumin is required to collect LPS from the solution. Stimulation of whole blood with the supernatant remaining after incubation of albumin-coupled beads with up to 800 pg/ml LPS led to no significant IL-1 β release (data not shown).

Fig. 1. Endotoxin concentration response curve in AWIPT. AWIPT was performed with a concentration series of LPS from 0 to 1600 pg/ml in 0.9 % saline. (A) Sigmoidal regression according to Boltzman ($r^2=0.86$). (B) Linear approximation over a limited concentration range (0–200 pg/ml) ($r^2=0.85$). Depicted data correspond to sextuples from one blood donor $\pm$ S.D. Cut-off: mean (blank) + 3 $\times$ S.D. $p < 0.05$, or $p < 0.01$ versus unstimulated control (Dunnett's multiple comparison).

Fig. 2. Comparison of LPS binding capacity of unmodified and albumin-coupled beads. Unmodified and albumin-coupled macroporous beads were pre-incubated with 25–100 pg/ml LPS in a volume of 100 μ l and then incubated with whole blood after washing. IL-1 β release was measured by ELISA. Data are means $\pm$ S.D. of triplicates from one blood donor.

3.3. Comparison of IPT and AWIPT with regard to LPS or LTA response

100 μ l samples of LPS (3 to 800 pg/ml) or LTA (1 to 100 μ g/ml) were subjected to IPT or AWIPT in parallel. Fig. 3 shows that IL-1 β secretion levels were generally higher in the case of the AWIPT, but that IL-1 β secretion became detectable upon stimulation with the same concentrations of LPS or LTA. Thus, although IL-1 β secretion was greater in the AWIPT, the sensitivity of pyrogen detection was comparable with that in IPT.

3.4. Kinetics of cytokine release in IPT versus AWIPT

The kinetics of cytokine release in IPT and AWIPT were compared. As shown in Fig. 4, cytokine release became detectable somewhat later in AWIPT in comparison to IPT. However, the cytokine levels attained for the same LPS concentration were again higher in AWIPT.

Fig. 3. Comparison of IL-1 β response to LPS and LTA in IPT and AWIPT. 100 μ l samples of 3 to 800 pg/ml LPS or 1 to 100 μ g/ml LTA were employed in parallel in IPT and AWIPT. IL-1 β release was measured by ELISA. Data represent means $\pm$ S.D. of triplicates from one blood donor.

Fig. 4. Kinetics of IL-1 β induction in whole blood test and AWIPT. Whole blood assay (IPT) and AWIPT were performed in parallel. Saline spiked with 200 pg/ml LPS was used as sample. For each time point and cytokine, a blank was measured as control (not shown). Data are depicted as means $\pm$ S.D. of duplicates of 2 blood donors.

3.5. Detection of endotoxin in complex therapeutics

The drug samples were diluted in series, and then spiked with LPS. These samples were employed in parallel in IPT and AWIPT. All drugs showed interference in the IPT at the highest concentrations used. Interference is overcome when the spike recovery reaches a value between 50% and 200% of the cytokine induction stimulated by the same amount of LPS in the absence of the drug (Hoffmann et al., 2005).

Table 1 shows that spike recovery was always achieved at lesser dilutions in AWIPT in comparison to IPT, except for the case of gentamicin spiked with 25 pg/ml LPS in which recovery was achieved at the same dilution in both tests. These results are illustrated for the example of liposomal daunorubicin in Fig. 5.

Considering the detection limit of the AWIPT, given by the cut-off value of the respective test, a positive signal in samples of these drugs at these dilutions would indicate that the stock solutions are contaminated with at

Fig. 5. Detection of endotoxin spikes in dilutions of liposomal daunorubicin. Liposomal daunorubicin (2 mg/ml) was diluted in series and spiked with 25 pg/ml LPS. Samples of these dilutions were employed in the IPT and the AWIPT in parallel. IL-1 β was measured by ELISA. Data are given as means $\pm$ S.D. of triplicates. 50% of cytokine secretion in response to the LPS spike are marked by a dashed horizontal line.

least that amount of LPS equivalent per ml multiplied by the dilution factor used in the test. The crucial contamination level that would induce a fever reaction in a patient is 5 EU per kg, i.e. 500 pg of the LPS used. Taking 70 kg as the average weight of an adult, 350 EU, equivalent to 35 ng LPS from *E. coli* O113, can be administered without adverse effects. Thus, the maximum daily dosage must be included in the calculation.

Using AWIPT, all drugs except cisplatin could be tested for pyrogenic contamination with a sufficient safety margin at concentrations at or below the maximal valid dilution. The maximum valid dilution is defined as the endotoxin limit concentration multiplied by the concentration of the drug in the test solution divided by the sensitivity limit of the assay. The endotoxin limit concentration is defined as the LPS threshold per kg (i.e. 500 pg LPS O113 or equivalent) divided by the maximum recommended drug dose per kg. The interference of cisplatin was reduced in the AWIPT in comparison to the IPT, although the safety margin was still overstepped by a factor of 3.

4. Discussion

The original In vitro Pyrogen Test protocol directly brings together the test sample with human whole blood and measures IL-1 β secretion in response to possible

Table 1
Minimum dilutions required for recovery of LPS spike in complex therapeutics

	IPT	AWIPT
Paclitaxel	1/100	1/10
Gentamicin	1/30	1/30
Cisplatin	1:100	1/10
Prednisolone	n.t.	1/10
Liposomal amphotericin B	1/10	1/3
Liposomal daunorubicin	n.t.	1/30

Drugs were diluted in series and spiked with 25 pg/ml LPS. Recovery of spike, defined as cytokine release corresponding (50–200%) to levels induced by the same concentration of LPS in the absence of the drug, was achieved at the given dilutions; n.t., not testable at dilutions up to 1/300.

pyrogenic contamination. As some drugs are toxic for blood cells or interfere with cytokine production, we adapted the IPT to the testing of such substances by including a prior adsorption step, in which possible pyrogenic contaminations can bind to albumin-coated microspheres, which are then separated from the interfering substance for stimulation of human whole blood.

The test shows a detection limit of 25 pg/ml LPS. With respect to endotoxin, the IPT is less sensitive than the LAL (detection limit of 3 pg/ml) and slightly more sensitive than the rabbit test (Hoffmann et al., 2005). Binding of LPS to the albumin beads is probably mediated by hydrophobic interactions (Jurgens et al., 2002), suggesting that lipopeptides or glycolipids may bind as well. Indeed, detection of LTA, a non-endotoxin pyrogen, was possible in the same concentrations as in the IPT. Importantly, the binding of the pyrogens is sufficiently strong to withstand several washing steps with Tween-20.

The cytokine release in the AWIPT was higher than that in the IPT at every LPS concentration employed, although the amount of blood was the same in both assays. This could indicate that the bound LPS is presented to the immune cells in an optimal manner by the albumin. The sensitivity of the assays was comparable. This is in line with the observation that the beads bound the LPS in the solution so efficiently that the remaining supernatant was not able to induce cytokine release.

The kinetics of cytokine release in response to the beads were somewhat delayed in comparison to LPS stimulation in the absence of beads. This may reflect a requirement for the LPS to dissociate from the albumin and be transferred to the cellular receptor. The higher cytokine release detected in AWIPT in comparison to IPT may in part be explained by the lower total incubation volume used for the AWIPT with the same amount of blood.

A selection of drugs known to interfere with the rabbit pyrogen test and in part also with the Limulus test, i.e. prednisolone (van Noordwijk and de Jong, 1977; Hartung et al., 2001), was used to test the efficiency of pyrogen detection by IPT and AWIPT. All drugs interfered with the IPT as expected. The AWIPT allowed spike recovery at a dilution at least 3 fold less than in IPT for all drugs except for gentamicin spiked with 25 pg/ml LPS where the recovery was the same in both tests. The safety margin required to exclude relevant pyrogenic contamination was attained for all drugs except cisplatin, where it was missed only by a factor of 3.

Importantly, endotoxin spikes were allowed to bind to the drugs for 2 h. This critical step is often omitted in the LAL protocols and results in an arbitrary spike recovery. The adsorption phase of the proposed test offers a unique opportunity for redistribution of bound endotoxin to beads with higher affinity.

All the drugs tested are known to bind to serum albumin. Some of the drugs affect the conformation of albumin and thus the binding of other molecules, and some may compete for binding with LPS (Trynda-Lemiesz and Luczkowski, 2004). Thus, how well the drug binds to the immobilized albumin and how this affects the activity of the drug, i.e. whether its activity is masked or potentiated when carried over to the whole blood incubation, and how the binding of the drug to albumin affects the affinity of the albumin molecules to bind LPS may all have a bearing on the result obtained in the AWIPT. This may explain why the AWIPT does not resolve the interference of all the drugs to the same degree. However, results obtained in the current study indicate that this theoretical interference does not occur to a relevant extent in the drugs studied. As this may be different for other similar drugs, every drug to be tested must initially undergo an interference test, i.e. a comparison of the cytokine release in blood induced by albumin beads exposed to an LPS spike in the presence and in the absence of the drug.

The use of a more specific pyrogen binding molecule, e.g. CD14, as decoration of the beads might reduce the interference of drugs even further as they are less likely to bind it. However, as the nature of many pyrogens is not yet known, it is not clear how this would limit the spectrum of detectable pyrogens.

Taken together, the inclusion of an adsorption and washing step into the IPT procedure improved the detection of pyrogenic contaminations in immunomodulatory and toxic drug samples. This modified procedure offers an improvement for the safety testing of products that are administered to patients with severe diseases. Application studies of this test for batch control of toxic or immunomodulatory medications are required to establish how commonly pyrogenic contaminations occur and to what extent some side effects may be attributed to them. Also, the applicability of the test to medications similar to those chosen here as prototypes must be determined.

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References

- Bhattacharya, A.A., Grune, T., Curry, S., 2000. Crystallographic analysis reveals common modes of binding of medium and long-chain fatty acids to human serum albumin. *J. Mol. Biol.* 303, 721.
- Cohen, J., 2000. The detection and interpretation of endotoxaemia. *Intensive Care Med.* 26 (Suppl. 1), S51.
- Deeter, L.B., Martin, L.W., Lipton, J.M., 1989. Age- and sex-related differences in febrile response to peripheral pyrogens in the rabbit. *Gerontology* 35, 297.
- Dehus, O., Hartung, T., Hermann, C., 2006. Pseudomonas endotoxin, a prominent contamination of water samples, is strongly overestimated in the Limulus Amebocyte Lysate Assay with regard to its pyrogenicity. *J. Endotoxin Res.* 36, 371.
- El-Khalik, A.F., Benoliel, D.M., 1982. Effects of weight and sex of rabbits on the results of the testing of chorionic gonadotrophin injection according to the European pharmacopoeia. *J. Pharm. Pharmacol.* 34, 209.
- Fennrich, S., Wendel, A., Hartung, T., 1999. New applications of the human whole blood pyrogen assay (PyroCheck). *Altex* 16, 146.
- Hartung, T., Wendel, A., 1995. Die Erfassung von Pyrogenen in einem humanen Vollblutmodell. *Altex* 12, 70.
- Hartung, T., Aaberge, I., Berthold, S., Carlin, G., Charton, E., Coecke, S., Fennrich, S., Fischer, M., Gommer, M., Halder, M., Haslov, K., Jahnke, M., Montag-Lessing, T., Poole, S., Schechtman, L., Wendel, A., Werner-Felmayer, G., 2001. Novel pyrogen tests based on the human fever reaction: the report and recommendations of ECVAM workshop 43. *Altern. Lab. Anim.* 29, 99.
- Hoffmann, S., Peterbauer, A., Schindler, S., Fennrich, S., Poole, S., Mistry, Y., Montag-Lessing, T., Spreitzer, I., Loschner, B., van Aalderen, M., Bos, R., Gommer, M., Nibbeling, R., Werner-Felmayer, G., Loitzl, P., Jungi, T., Brcic, M., Brugger, P., Frey, E., Bowe, G., Casado, J., Coecke, S., de Lange, J., Mogster, B., Naess, L.M., Aaberge, I.S., Wendel, A., Hartung, T., 2005. International validation of novel pyrogen tests based on human monocytoïd cells. *J. Immunol. Methods* 298, 161.
- Hull, D., McIntyre, J., Vintér, J., 1993. Age-related changes in endotoxin sensitivity and the febrile response of newborn rabbits. *Biol. Neonate* 63, 370.
- Jurgens, G., Muller, M., Garidel, P., Koch, M.H., Nakakubo, H., Blume, A., Brandenburg, K., 2002. Investigation into the interaction of recombinant human serum albumin with Re-lipopolysaccharide and lipid A. *J. Endotoxin Res.* 8, 115.
- Martis, L., Patel, M., Giertych, J., Mongoven, J., Taminne, M., Perrier, M.A., Mendoza, O., Goud, N., Costigan, A., Denjoy, N., Verger, C., Owen Jr., W.F., 2005. Aseptic peritonitis due to peptidoglycan contamination of pharmacopoeia standard dialysis solution. *Lancet* 365, 588.
- Morath, S., Geyer, A., Hartung, T., 2001. Structure-function relationship of cytokine induction by lipoteichoic acid from *Staphylococcus aureus*. *J. Exp. Med.* 193, 393.
- Petitpas, I., Grune, T., Bhattacharya, A.A., Curry, S., 2001. Crystal structures of human serum albumin complexed with monounsaturated and polyunsaturated fatty acids. *J. Mol. Biol.* 314, 955.
- Poole, S., Mussett, M.V., 1989. The International Standard for Endotoxin: evaluation in an international collaborative study. *J. Biol. Stand.* 17, 161.
- Schindler, S., Fennrich, S., Crameri, R., Jungi, T., Montag, T., Hartung, T., 2001. Fever in the test tube—towards a human(e) pyrogen test. *3R-Info.-Bull.* 1.
- Schindler, S., Bristow, A., Cartmell, T., Hartung, T., Fennrich, S., 2003. Comparison of the reactivity of human and rabbit blood towards pyrogenic stimuli. *Altex* 20, 59.
- Staubach, K.H., Boehme, M., Zimmermann, M., Otto, V., 2003. A new endotoxin adsorption device in Gram-negative sepsis: use of immobilized albumin with the MATISSE adsorber. *Transfus. Apher. Sci.* 29, 93.
- Trynda-Lemiesz, L., Luczkowski, M., 2004. Human serum albumin: spectroscopic studies of the paclitaxel binding and proximity relationships with cisplatin and adriamycin. *J. Inorg. Biochem.* 98, 1851.
- Ullrich, H., Jakob, W., Frohlich, D., Rothe, G., Prasser, C., Drobnik, W., Taeger, K., Meier-Hellmann, A., Reinhart, K., Zimmermann, M., Schmitz, G., 2001. A new endotoxin adsorber: first clinical application. *Therap. Apher.* 5, 326.
- van Noordwijk, J., de Jong, Y., 1977. Comparison of the Limulus amebocyte lysate (LAL) test with the rabbit test: false positives and false negatives. *Dev. Biol. Stand.* 34, 39.
- Zimmermann, M., 2000. In vitro Untersuchungen zum Adsorptionsverhalten eines Endotoxinadsorbers auf der Basis von immobilisiertem Humanalbumin. In PhD thesis, University of Saarbrücken.
- Zimmermann, M., Busch, K., Kuhn, S., Zeppezauer, M., 1999. Endotoxin adsorbent based on immobilized human serum albumin. *Clin. Chem. Lab. Med.* 37, 373.