Immunotherapy of Severe Sepsis and Septic Shock: Is there a Future?

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Introduction

Sepsis syndrome [1] and septic shock are always associated with a high rate of mortality. It has been estimated that in Western Europe, 400 000 to 500 000 cases of sepsis syndrome are diagnosed each year with 40 to 70% of the patients developing septic shock. Mortality is at approximately 40% in cases of sepsis syndrome with gram-negative bacteremia [2–4], 50% with hypotension [2–5] and can reach 70–90% in cases of shock with multiple organ failure (MOF) [2–7]. The pathogenic and physiopathologic complexity of sepsis syndrome can, in large part, explain the difficulties encountered in establishing therapeutic strategies. The number of mediators and cells are unlimited (Fig. 1) and new mediators are regularly isolated (adhesion molecules, endothelin-1 ... !).

Some elements of the therapeutic strategy for sepsis syndrome have not been called into question:

- aggressive treatment of the infection (association of antibiotics, surgery, draining of abscesses ...),
- hemodynamic care adapted to the type of cardiovascular problem observed,
- general supportive care measures (artificial nutrition, mechanical ventilation ...).

However, given the persistent high rate of mortality, other therapeutic solutions must be considered. Their aim would be to neutralize the effects of the various mediators in question.

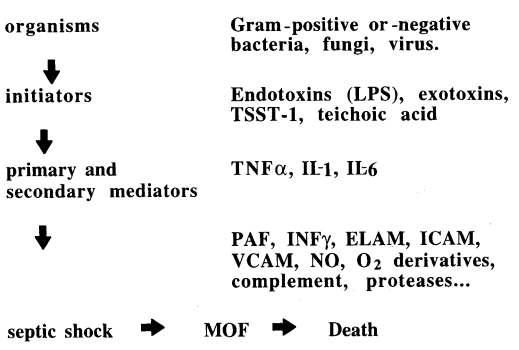

Fig. 1. Pathophysiology of severe sepsis and septic shock

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Table 1. How to interfere with cytokines?

Mechanisms	Drugs
- Monoclonal antibodies	- anti TNF-α - anti IL-1, IL-2, IL-6, ... - anti INF γ
- Soluble receptors	- to TNF-α, IL-1, IL-6, INF γ
- Antagonism at the receptor level	- IL-1ra - antibody of TNF-α receptor
- Inhibition of production (synthasis/release)	?
- Increased clearance	?
- Interference with post-receptor effects: modulation of post-signal transduction	?

Anti-Endotoxin Therapies

Gram-negative bacteria continue to be a frequent cause of sepsis syndrome and its complications. Endotoxins, which are normal components of the walls of these germs are capable of initiating a cascade of events that leads to sepsis syndrome. The activation of macrophage cells permits the liberation of tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2, IL-6, platelet activating factor (PAF) and other mediators (Table 1). Endotoxins also have a direct role on the complement and coagulation cascades. The central role of endotoxin has also been demonstrated by the reproduction of sepsis symptoms following administration in animals or volunteers [8]. Moreover, it has been known for a long time that sepsis is more severe and deaths more frequent when low levels of antigen O IgG or antipolysaccharide IgM are detected in the circulating blood [9, 10]. This has naturally led to attempts to increase the concentrations of such antibodies in patients presenting sepsis syndrome.

Utilization of Natural Polyclonal Anti-Endotoxin Antibodies

Certain patients naturally possess high levels (>40 μg/mL) of various IgG polyclonal antibodies which can bind to the endotoxins of numerous germs: *E. coli, Klebsiella, Proteus, Pseudomonas* ... [11]. With such donors, a hyperimmune serum became available. It was rich in antibodies and was used by different authors [11, 12]. In such cases, the patients were in a state of septic shock, at times very severe [12], and receiving this treatment as part of an open study without controls. These authors all reported a very favorable impression but, owing to methodological problems, it is very difficult to make any conclusions. One study [13] reported the use of a special preparation of IgM-enriched immunoglobulins that made it possible to significantly reduce mortality when compared with the control group. This type of preparation (Pentaglobulin) can possibly be recommended in cases of septic shock.

Utilization of Polyclonal Antibodies to the Core of Endotoxin

Endotoxin is a very complex structure which is roughly made up of three parts: a lateral chain composed of a row of oligosaccharide units (O antigen), a core (polysaccharide), and lipid A (considered as the active part). Natural anti-endotoxin antibodies are directed against the O antigen and are thus very specific to one species. They can only provide protection for one type of bacterial strain. The central part of the endotoxin (core + lipid A) is a structure that varies little from one bacterium to another [9]. The utilization of an antibody against this region of the endotoxin is efficient for a great number of gram-negative bacteria. Certain mutant bacteria have lost their ability to synthesize the oligosaccharide chain because they no longer possess the epimerase-type enzyme required for the incorporation of galactose. Thus, the lateral chain can no longer fix itself to the core of the endotoxin. From these strains (*Salmonella minnesota* S-128 or Re 595, and especially J5 mutants of *Escherichia coli* 0111:B4) which were inactivated by heat, some authors have vaccinated healthy volunteers and obtained a production of high titers of polyclonal endotoxin anticore IgG antibodies. These polyclonal antibodies have been used in the form of plasma, serum or purified preparations of IgG in various studies.

Analysis of J5 studies with polyclonal antibodies: Six studies have been performed with this type of IgG antibody directed against the core of the endotoxin [3, 14–18]. Of three studies that evaluated the effects of curative treatment, only one can be considered as a success. The study, published by Ziegler et al. in 1982 [3], included 304 patients and demonstrated that J5 antiserum reduced the mortality of patients with a gram-negative bacteremia from 38 to 24% ($p < 0.041$). The mortality of patients with bacteremia and hypotension went from 52 to 32% ($p < 0.028$), and patients with severe septic shock (vasopressors for more than 6 h) presented a mortality rate that decreased from 76 to 46% ($p < 0.009$). Moreover, this study showed that patients who did not present bacteremia did not benefit from treatment with J5 antiserum. Finally, the results did not make it possible to make any conclusions for patients presenting with septic shock with negative blood cultures.

The study by Baumgartner et al. [14] has demonstrated the interest of a prophylactic treatment by plasma containing anti J5 IgG polyclonal antibodies administered to patients undergoing surgery with a high risk of infectious complications. The incidence of postoperative gram-negative bacilli (GNB) infections was not reduced. On the other hand, the incidence and the mortality of postoperative septic shock were very significantly reduced.

The four other studies were considered as failed [15–18]. Two of them are discussed here. In the study by Calandra et al. [15], a preparation enriched in endotoxin anticore immunoglobulin G (obtained after vaccination of volunteers by the J5 mutant) did not reduce the mortality of patients with septic shock (IgG standard: mortality 50%, IgG anti J5: mortality 49%). The study concerning the treatment of purpura fulminans by an anti J5 plasma was prematurely interrupted after the inclusion of 73 children because neither evolu-

tion nor mortality was modified by the treatment [16]. Mortality was 36% in the control group versus 25% in the J5 group (a difference of 11% with a 95% confidence interval from 10 to 32%: $p = 0.32$). However, the low number of patients did not permit the authors of this study to detect the significance of a slight difference in mortality between the groups.

An analysis of the 6 available studies can only lead to disappointing conclusions. Among the curative studies, only one was successful. The failures of the studies that used immunoglobulin preparations enriched in endotoxin anticore antibodies can be explained by the destruction or the denaturation of the antibodies during industrial preparation, or by the lack of IgM, which would seem to be the most active antibody. Moreover, there are numerous reasons for abandoning the use of serum or plasma:

- toxicity (low but indisputable) in the volunteers for vaccination by *E. Coli* J5 mutants,
- difficulty in obtaining standardized preparations,
- storage difficulties,
- major risk of transmission of viral diseases.

All of this led to the industrial development of monoclonal antibodies.

Utilization of Industrial Endotoxin Anticore Monoclonal Antibodies [19]

The utilization of biotechnologies makes it possible to produce great quantities of monoclonal antibodies with an isotype that is perfectly known and specific to an epitope. In addition, the risks of infection are eliminated by these techniques which provide antibodies that are very purified and sterilized. Two anticore endotoxin IgM antibodies have been recently studied (Table 2). Both are obtained from cultures of cellular lines. The same strain of J5 *E. coli,* which had permitted the production of the previously discussed polyclonal antibodies, was used as an immunogen for the stimulation of the cells. Both antibodies were studied in prospective, controlled, randomized, double-blind studies.

Centoxin (HA-1A) (Centocor, Malvern, PA, USA): The first HA-1A study included 543 patients presenting severe sepsis with or without septic shock [4]. The

Table 2. Monoclonal anti-endotoxin antibodies

HA-1A (Centotoxin®, Centocor, Malvern, PA, USA)	E5 (Xomen™, Xoma Corporation, Berkeley, CA, USA)
- IgM, human origin	- IgM, murine origin
- Immunogen: J5 *E. coli*	- Immunogen: JE *E. coli*
- Heteromyelomatous cell line A6-H4C5	- Mouse ascitis
- Single dose: 100 mg	- Two doses: 2 mg/kg, 24 h apart

subgroup targeted by the study was, following the 1982 study by Ziegler et al. [3], that of patients presenting a gram-negative bacteremia. In this subgroup of 200 patients (37% of the total), mortality was significantly reduced on the 28th day from 49 to 30%. This very significant reduction was also found for bacteremic patients with septic shock (mortality reduced from 57 to 33%) including the forms of shock with organ failure (mortality reduced from 73 to 36%). However, no benefit was found for the 201 patients with sepsis syndrome with gram-negative bacilli (GNB) without bacteremia, nor for the 142 patients presenting with sepsis of another origin. Antibody tolerance was excellent and no anti HA-1A antibody was detected at the end of the study. This study by Ziegler et al. [4] has been the object of a heated debate and its clinical implications have been greatly disputed [3, 19–25]. One strong criticism has been of the poor matching between the two groups. For several important prognostic factors (acute renal failure, APACHE II score, disseminated intravascular coagulation, ARDS), the placebo group was disadvantaged (but not significantly), which could have influenced the results.

A second double-blind, randomized study was therefore undertaken and stopped after the inclusion of 2199 patients with septic shock (Chess study: Centocor HA-1A Efficacy in Septic Shock) [26]. This study was prematurely interrupted because of a low extramortality of patients not presenting a GNB infection: 42.3% or 244/577 patients as opposed to 37.8% or 230/608 patients in the control group ($p = 0.134$). When the study was stopped, the mortality rate of the 328 patients presenting a GNB infection was at 33% (109/328) versus 32% in the control group (95/293) ($p = 0.86$). This second study, therefore, did not confirm the results of the first.

A third publication evaluated the usefulness of HA-1A monoclonal antibodies in a cohort study of 600 patients with septic shock [27]. The mortality of this group (61.3%) turned out to be slightly superior to that predicted by APACHE II score (56%, $p = 0.03$). The authors of this study concluded that the patients presenting with septic shock with GNB bacteremia did not benefit from a treatment by HA-1A antibodies and suggest that this drug could have deleterious effects when the infection is not due to GNB.

Finally, the 543 patients in the Ziegler study were evaluated in order to determine if the use of HA-1A antibodies had had marked hemodynamic effects after their injection. A total of 197 patients were equipped with a Swan-Ganz catheter [28]. The authors found no relationship between hemodynamic modifications, the presence of a GNB bacteremia, or the utilization of the HA-1A antibodies. This evaluation was retrospective, with all of the methodological limitations that are inherent to this type of *a posteriori* analysis.

E5 Studies (Xoma Corporation, Berkeley, CA, USA): The first study included 486 patients with severe sepsis with or without septic shock [29]. The aims of the study did not define which subgroups of patients were supposed to benefit from the treatment. The results show that a reduction in mortality was obtained on the 30th day in the subgroup of 137 patients with gram-negative sepsis without shock (mortality reduced from 43 to 30%). The presence or absence of bacteremia did not influence the results. The authors observed no

reduction in mortality in the 179 patients presenting GNB infection accompanied by septic shock, nor in the 152 patients presenting sepsis not linked to a gram-negative bacterium. However in the subgroup of 137 patients with GNB sepsis and without septic shock, survival at 30 days was 70% in the E5 group as opposed to 57% in the control group ($p = 0.01$). This was an *a posteriori* analysis [29]. Tolerance of the E5 antibody was very good with only 4 cases of allergic reaction, but 47% of the patients presented anti-E5 antibodies of a murine origin (mouse) in their serum.

The second study with E5 antibodies included 847 patients presenting the same criteria as in the previous study [30, 31]. In the group (in which prognosis was improved in the first E5 study) of 530 patients with GNB sepsis without shock, mortality was not significantly modified this time (E5: 30%, placebo: 26%). This unexpected failure led the authors to an analysis (*a posteriori*) of the subgroups where the treatment had been efficient: in cases of GNB sepsis, with and without shock, but with organ failure!!! This study therefore casts some doubt on the efficacy of E5 antibodies.

The third E5 study was undertaken on a small population of patients suspected of developing GNB sepsis. Mortality was at 70% in the E5 group and 73% in the placebo group (not significant) [32]. Moreover, 8 of the 15 patients who were given the E5 antibodies developed anti-E5 antibodies [32].

Reflections on the use of anti-endotoxin antibodies: In the last 15 years, 12 prospective, randomized, double-blind studies have evaluated the potential therapeutic role of anti-endotoxin antibodies administered for prophylactic or curative reasons for GNB infections [3, 4, 14–18, 26, 27, 29, 30, 32]. Six studies [4, 26, 27, 29, 30, 32] involved the use of antilipid A endotoxin monoclonal antibodies (HA-1A-Centoxin, and E5-Xomen, E5). Whereas the results of the first studies showed a benefit in certain subgroups, these results were not confirmed in subsequent studies. This led to withdrawal of Centoxin, from the market and Xomen, E5 was not put on the market. Further studies with other antibodies that are more specific and efficient should be undertaken in animals.

Other Anti-Endotoxin Therapies

Circulating endotoxin in the plasma binds to a glycoprotein of 60 kDaltons of an hepatic origin with levels that range from 0.5 to 50 μg/mL. The protein (lipopolysaccharide binding protein, LBP) has a high affinity for endotoxin and the LPS-LBP complex is then fixed by means of different membrane receptors (CD14, CD11/CD18, p 73 and others) onto the macrophage cells. The result is the synthesis and liberation of TNF-α and interleukin(IL)-1, IL-2, and IL-6. In order to block this cascade of events, antireceptor monoclonal antibodies CD14 and p 73 were developed. In animals submitted to an injection of endotoxin, a reduction in the production of TNF-α and IL-1 and an improvement in survival were observed [33].

Another research approach consists in using LPS competitive antagonists on the receptors. Lipid X is a precursor of lipid A in certain mutant strains of *E. coli.* This precursor is much less toxic and competes with lipid A for fixation onto membrane receptors. It is capable of protecting mice from the lethal effects of an injection of endotoxin [53].

A natural protein, originally isolated from polynuclear azurophile granulations, neutralizes the endotoxin by fixing onto it with a very strong affinity. This bactericidal permeability increasing protein (BPI) has an affinity for endotoxin that is considerably higher than that of the HA-1A and E5 antibodies. The failure of these antibodies in clinical studies can in part be explained by their affinity for endotoxin which is too low. This new product therefore holds great potential interest. Moreover, BPI has proven to be experimentally efficient in reducing mortality following injection of endotoxin in rat and mouse [36]. Human studies must now be performed in order to evaluate its therapeutic interest.

Anti-TNF-α Therapies

TNF-α is a polypeptide that is essentially secreted by macrophages. It is capable of inducing a multitude of effects that are found in sepsis syndrome and septic shock situations. TNF-α is often considered as one of the central mediators in the physiopathogenesis of septic shock.

- TNF-α levels are high in a great number of sepsis syndromes [37–39];
- An injection of endotoxin triggers an increase in the circulating levels of TNF-α including in man [8, 37];
- An injection of TNF-α reproduces the signs and symptoms of severe sepsis [8, 37–43];
- TNF-α is liberated by a number of infectious agents: gram-positive and gram-negative bacteria, viruses, parasites, fungi [37, 39–43].

Anti-TNF-α Monoclonal Antibodies (MAb)

Anti-TNF-α MAb were developed for therapeutic purposes. They are capable of protecting animals from the lethal effects of an injection of endotoxin, whether it is administered after [42, 44–46] or even before [45, 47, 48] the utilization of anti-TNF-α antibodies. Anti-TNF-α antibodies have also proven their efficacy by protecting animals submitted to gram-negative sepsis. This has been demonstrated in animal models using higher primates (baboons) [49]. However, in a model of gram-positive infection in mouse, the anti-TNF-α antibodies were not efficient whereas the opposite had been the case for gram-negative sepsis [50]. In view of all of these elements, including the central role of TNF-α in the development of sepsis, interfering with this cytokine in order to minimize its effects would appear to be a promising therapeutic approach. In addition, other solutions that use MAb could be considered (Table 1). However, it should be noted that the studies considered above respond to a

precise experimental model: the injection of a single dose of endotoxin. This hardly corresponds to what is observed in man where endotoxin levels remain high for several hours (days) and where one does not observe a peak of short duration of TNF-α, but levels that remain consistently high.

In order to study the effects of anti-TNF-α MAb in conditions that are closer to clinical reality, animal models that stimulate peritonitis have been developed. A sepsis of prolonged duration with persistent levels of TNF-α was subsequently obtained. In such experimental models, the efficacy of anti-TNF-α antibodies is much less clear, even when they are associated with an antibiotherapy, and the groups treated by anti-TNF-α do not have a different prognosis than the control groups [51, 52]. Nevertheless, in a model of this type, anti-TNF-α associated with gentamicin was capable of considerably reducing mortality [53]. Certain other experimental models must also be considered with caution versus the efficacy of an anti-TNF-α antibody. In a neutropenic rat model, for example [54], the utilization of anti-TNF-α antibodies changed survival from 0 to 53% after creation of a *Pseudomonas* infection. This confirmed what was already known. Another interesting point is that the association of anti-TNF-α plus an antibiotherapy with ciprofloxacin pushed survival up to 100%. This confirms what clinicians were intuitively thinking: antibiotherapy retains a certain usefulness for the treatment of sepsis syndrome! But this same study showed that the animals treated with ciprofloxacin alone had a survival rate of 67%! Better than the anti-TNF-α! Extrapolation in man is difficult, however, because a survival of 67% with antibiotherapy alone has unfortunately not been found in man within the framework of septic shock ...

Interpretation of the manipulation or TNF-α levels has been made even more difficult by the results of studies showing that an antiendotoxin antibody is beneficial to survival when the levels of TNF-α have not been changed [55, 56], or when the considerable decrease of TNF-α obtained after the use of an anti-TNF-α antibody does not change survival in certain experimental endotoxinemia models [55, 56].

In man, several clinical studies have evaluated the use of anti-TNF-α antibodies (Table 3). Murine antibody CB0006 (Celltech Inc.) was submitted to Phase I and Phase II clinical trials [57, 58]. Its development was stopped because of its very strong immunogenicity. The humanized antibody CDP571 (Celltech Inc.) was only subjected to a very short Phase II trial [59]. This was also the case for cA2 (Centocor) [60] and MAK195F (Knoll) [61, 62]. There have been two large Phase III trials for murine antibody Bay X 1351 (Norasept I and Intersept) [63–65]. In the Norasept I study, only the subgroup of patients with shock seemed to benefit with a non-significant decrease of 17% in mortality (37.7 versus 45.6% in the control group, $p = 0.15$). No benefit, but a slight extramortality (24.8 versus 21.1%), was observed in the group of patients without shock [63, 65]. The study was interrupted for the patients without shock and continued for those in a state of septic shock (Norasept II). The Intersept study, conducted with the same antibody in 553 patients came to similar conclusions [64].

Table 3. Clinical evaluation of anti-TNF-αMAb

Antibody	Study	Comments	Ref
Murine antibody (CB0006, Celltech)	- Phase I - 14 patients - pre-mortem septic shock	Very good tolerance	[57]
	- Phase II 80 patients - severe sepsis	Potential benefit if high TNF serum levels	[58]
Murine antibody (Bay X 1351, Bayer/Miles)	- Norasept I - Phase III 994 patients sepsis severe	Very good tolerance. Trend toward reduced mortality in septic shock patients.	[63]
	- Intersept I - Phase III 553 patients - severe sepsis	Very good tolerance. Trend toward reduced mortality in septic shock patients.	[64]
	- Norasept II - Phase II (on going) septic shock	Very good tolerance.	
Humanized antibody (CDP 571, Celltech)	- Phase II 42 patients - septic shock	Very good tolerance	[59]
F (ab')2 fragment (MAK 195F, Knoll)	- Phase II 122 patients severe sepsis	Very good tolerance Trend toward reduced mortality if high IL-6-serum levels	[62]
Humanized antibody (CA_2, Centocor)	- Phase I/II 141 patients, severe sepsis	Very good tolerance	[60]

TNF-α Soluble Receptors

TNF-α soluble receptors are circulating natural inhibitors of TNF-α that come from the proteolytic cleavage of cellular receptors. In order to prolong the serum half-life, the molecules were synthesized into 2 groups of extracellular receptors binding covalently to the FC fragment of an IgG molecule. As there are (at least) two types of TNF-α receptors (55 kDA and 75 kDA), two types of soluble receptors are synthesized. The first (rs TNFR-IgG, Immunex) was submitted to a very disappointing Phase II study in 141 patients [66]. Two groups treated with the soluble receptor (0.45 and 1.5 mg/kg) presented an extramortality on the 28th day (48 and 53%) when compared with the control group (30%)! The group treated with 0.15 mg/kg presented mortality that was equal (30%) to that of the control group. It is possible that the circulating TNF-α remained stored on the soluble receptor molecules, that elimination of the organism could not occur, and that the TNF-α was released late in the evolution leading to a delayed serum peak and death of the patients! A new soluble

receptor is currently under evaluation. It is made up of the protein from 55 kDA (rs TNFR-p 55, RO 45 - 2088, Hoffmann-La Roche). A study that will include 200 patients is under way.

A few Thoughts concerning anti-TNF-α Strategy

- TNF-α is a molecule that nature has developed in almost all living species. Its role is to amplify the normal defense mechanisms of the organism against aggressions. Only an explosive reaction that spreads throughout the organism would seem to have negative effects ...) [67].
- It would therefore seem necessary to limit the systemic effects of TNF-α while at the same time preserving its local effects (paracrine action).
- TNF-α is not found in all cases of sepsis, meaning that anti-TNF-α could be administered too soon ... or too late ...!
- Anti-TNF-α antibodies have not been efficient for all types of experimental sepsis [51]. It will be necessary to wait until this is the case in man.
- The use of experimental associations of antibodies must certainly be considered given the complexity of sepsis syndrome pathogeny, and in animal, an anti-LPS antibody associated with an anti-TNF-α antibody was more efficient than each used separately [54].
- Taken together, TNF-α is a mediator for immunologic and inflammatory reactions.

The "good" effects of TNF-α are those which are found to counteract granulomatous-type infections (tuberculosis, leishmaniasis) and infections due to intracellular bacteria (listeriosis, legionellosis). The " bad" effects of TNF-α are only due to a poorly controlled reaction, and from then on, one should not be surprised that a therapy is deleterious if it is prescribed at an inappropriate dose or period.

Other anti-TNF-α Therapies

In addition to MAb, there are numerous other substances that can interfere with TNF-α, generally by inhibiting its synthesis and liberation:
- corticoids (which are among the most active products)
- PGE_2
- pentoxifylline
- chloroquine
- theophylline
- PAF antagonists
- ethanol
- lactulose
- ...!

They are awaiting evaluation ...!

Anti-IL-1 Therapies

IL-1 is another polypeptide secreted by numerous cells which has numerous similarities with TNF-α. Like TNF-α, IL-1 plays a major role in the development of sepsis. IL-1 levels are high beginning at 3 to 4 h after the appearance of endotoxin, and they remain much longer than those of TNF-α (24 h or more) [20]. In animal and man, the administration of IL-1 reproduces signs of severe sepsis [33]. Theoretically, one could therefore conceive that any molecule that can interfere with the activity of IL-1 could potentially be of interest in the treatment of sepsis. There is a natural protein from 23 to 26 kD that shares 41% of the structure of IL-1β and 30% of that of IL-1α. This protein, which was formerly called "IL-1 inhibitor", is produced by the monocytes. Its role is to inhibit the fixation of IL-1 onto its receptor membranes, thereby preventing cellular activation [68]. Named "IL-1 receptor antagonist" or IL-1ra, this molecule has been synthesized by recombination and used in various septic shock models. The results have been very interesting (decrease in production of TNF-α, IL-1, IL-6, GM-CSF, nitric oxide) with, as a clinical corollary, reversion of arterial hypotension, leukopenia, an increase in cardiac index, and an improvement in survival [33, 47, 69, 70]. It is of interest to note that IL-1ra is efficient even if TNF-α levels are not high, which once more shows the great complexity of the mechanisms involved in the genesis of sepsis syndrome and the difficulty in understanding experimental models. Like anti-TNF-α antibodies, IL-1ra could be active in cases of sepsis due to gram-negative and gram-positive bacteria.

A Phase II study was undertaken with IL-1ra (Antril, Synergen Inc., Boulder, CO, USA) in 99 patients with severe sepsis. The results suggest that there was a dose-dependent reduction in mortality (control group: 44%, IL-1ra groups: 32, 25 and 18% for doses that ranged from 17 to 133 mg/h) [71]. Following these observations, a large Phase III study was conducted in 893 patients presenting severe sepsis. Once again, the results were very disappointing with 34% mortality in the control group versus 31% for a dose of 1 mg/kg/h and 29% for a dose of 2 mg/kg/h of IL-1ra. In this study, 713 patients presented septic shock, but here also, mortality was not influenced by the treatment ($p = 0.23$) [72]. An *a posteriori* analysis of the study made it possible to identify a subgroup of patients that benefited from the treatment: patients presenting a predicted mortality of $\geq 24\%$ and who received a dose of 2 mg/kg/h of IL-1ra. A new study was undertaken in this subgroup of patients using the same IL-1ra. A total of 700 patients were included but no benefit was found. In fact, the difference in mortality between the two groups was too low for the study to be worth continuing (Synergen News Release: Synergen stops clinical trial of Antril, for severe sepsis. July 18, 1994, Synergen Inc., Boulder, CO).

Therapies that interfere with other Cytokines

TNF-α and IL-1 are not the only cytokines that are involved in the pathogenicity of sepsis syndrome. This is the case for INF-γ, IL-6, IL-8, but the role of

IL-4 is less clear. An anti-IL-6 antibody was successfully tested in animal, improving the rate of survival after injection of *E. coli* or TNF-α. However, this antibody only appears to be efficient if administered before the induction of sepsis which will considerably limit its interest [73]. It is of interest to note that injection of the anti-IL-6 antibody was responsible for a net increase in TNF-α levels, contrasting its very beneficial effects on survival. This again raises the question of the role of TNF-α in the severity of sepsis syndrome. A change in INF-γ levels was also obtained in an animal study [74] with very favorable results for survival when an anti-INF-γ antibody was administered within 2 h of endotoxin injection. In the same study, the use of an anti-IL-4 antibody did not prove to be efficient. IL-4, IL-10 and TGF-β are anti-inflammatory cytokines that inhibit the secretion of TNF-α and IL-1-type inflammatory cytokines. They have demonstrated their ability to protect mice from the lethal effect of an injection of endotoxin while at the same time inhibiting TNF-α.

Given the importance of cytokines in the pathogenicity of septic shock, other therapies which use MAb could be considered (Table 1).

Therapies that interfere with PAF

PAF is a phospholipid obtained after the action of phospholipase A2 on 3-phosphocholine. Acetyl transferase then makes it possible to obtain PAF from lyso-PAF. This mediator has numerous activities that can explain its role in the pathogenicity of septic shock. A great number of PAF receptor antagonists are available (over 15 have been tested for experimental septic shock). These products are generally efficient in correcting arterial hypotension, thrombopenia, plasma extravasation, the liberation of ecosanoids, metabolic acidosis ...

Following these observations, a Phase III study was undertaken with BN 52021 (Ipsen-Beaufour) in 262 patients presenting severe sepsis. No reduction in mortality was observed for the group as a whole following administration of the anti-PAF. However, an *a posteriori* analysis showed a 42% reduction in mortality (control group: 57% versus anti-PAF group: 33%) in 119 patients presenting a documented gram-negative infection. A second Phase II study included 608 patients with severe sepsis, possibly of GNB origin. On day 28, no significant reduction in mortality had been observed [75]. Another analog of PAF, BB 882 (British Biotech) is also currently under evaluation.

Other Approaches

At present, a great many other therapeutic possibilities are also being considered (Table 4). None of them has provided definite proof of efficacy, but the future is certainly full of promise for the rational treatment of sepsis syndrome.

Table 4. Potential therapeutic strategies for severe septic states and septic shock

Antiendotoxin treatments	Anticytokine treatments
- HA1A (Centocor) - E5 (Xoma/Pfizer) - P88 (Chiron) - PBI (Xoma) - BPI/LBP (Incyte) - Antireceptor 14/s CD14 (Incyte/Eisair) - Lipid A analog (Ribi Immunochem)	- TNF antibodies (Bayer/Miles, Centocor, Celltech, Knoll) - Soluble TNF receptor (Immunex, Hoffmann-La Roche) - IL-1ra (Synergen) - Soluble IL-1 receptor (Immunex, Affymax) - IL-10 (Schering-Plough)
Anti-adhesion molecules	**Others**
- Anti E - selectin antibody (Cytel) - Anti CD11/CD18 antibody (Genetech, Repligen/Lilly) - Polymorphonuclear adhesion antagonists (Liposome Company)	- PAF antagonists (Beaufour, British Biotech, Takeda ...) - Prostaglandin E (Upjohn) - Leukotrienes inhibitors (Lilly) - Anti-elastase (Athena) - N-acetylcysteine (Zambon) - Pentoxyfilline - .....!!!

Conclusion

Many studies have been undertaken in order to determine the interest of modifying the immuno-inflammatory cascade during sepsis syndrome. So far, no Phase III study has given favorable results for the study populations as a whole. Consequently, it is at this time impossible to recommend any one therapeutic approach. Too many unknowns persist, in particular, is it preferable to simultaneously block the actions of several mediators (TNF-α, IL-1, PAF)? Then, is there no danger in totally blocking the response of the immune system? The clinical trial with the TNF-α soluble receptor is an example of the extramortality observed in a group of patients. New studies that use more adapted means of investigation are required.

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