An Historical Review of Glucocorticoid Treatment in Sepsis. Disease Pathophysiology and the Design of Treatment Investigation

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Abstract. This review provides an historical perspective on events that have shaped our pathophysiological understanding of sepsis during this century and, as a result, have influenced the design of glucocorticoid treatment trials. A structured format to evaluate clinical investigation of glucocorticoid treatment in patients with sepsis is presented. From the early 1950s to the late 1980s, the methodological quality of randomized trials improved, while the science dictating the design of treatment protocols overlooked prior clinical observations and relied almost exclusively on the findings of an experimental model that misrepresented human sepsis. As a result, the daily glucocorticoid dose escalated up to 140 fold while the duration of treatment decreased to 24-hours or less. Bedside observation was the foundation for evaluating treatment response in the early studies, while in the later trials, survival was the principle outcome measure and these reports frequently did not include serial recordings of biological, clinical, and physiological variables over time. Today, we appreciate that excessive activation of the host defense response in patients with septic shock or acute respiratory distress syndrome (ARDS) may induce noncompensated glucocorticoid resistance in target organs, thereby negating the beneficial suppressive influence of an inadequately secreted endogenous cortisol. Recent studies have shown that a dysregulated host defense response is characterized by exaggerated and protracted immune cells activation of nuclear factor- κ B and persistent elevation of biological markers (inflammatory cytokine levels, etc.) of disease activity over time. Within this new pathophysiological understanding of sepsis, prolonged treatment with increased doses of exogenous glucocorticoids may in theory modify the intracellular balance among activated transcription factors, and convert an initially dysregulated host defense response into a regulated one. Supporting evidence is provided by the results of recent controlled and uncontrolled studies of patients with septic shock and unresolving ARDS showing that prolonged glucocorticoid treatment was associated with a rapid, significant, and sustained reduction in circulating levels of markers of disease activity, and progressive physiological improvement. No other treatment intervention in sepsis or ARDS has yet provided this level of evidence.

Key Words. Acute respiratory distress syndrome, complications, disease, glucocorticoids, glucocorticoid receptor, infection, sepsis, syndrome, transcription factors, treatment

"It is only a knowledge of patho(physio)logy that enables us to devise rational therapy that can best heal the patient."

Giovanni Battista Morgagni: *Seats and Causes of Diseases*. Padua University, 1761.

Introduction

Today, there is renewed interest in the topic of glucocorticoid therapy in patients with septic shock and ARDS. Four small double-blind, randomized trials evaluating prolonged glucocorticoid therapy in patients with septic shock or unresolving ARDS (sepsis-induced) were recently completed [1-4]. In all four, prolonged glucocorticoid treatment was associated with a rapid and significant clinical improvement. In two of them, survival was an end-point and both reported a significant reduction in mortality [1,4]. Such significant results probably reflect a medium to large beneficial effect of therapy, although there is some concern that the small sample size may be insufficient to confidently evaluate an outcome benefit. The findings of these recent studies are in apparent contradiction with the results of previous randomized, multicenter trials investigating massive doses of glucocorticoid therapy in sepsis and ARDS [5-10]. Two recent meta-analyses carefully reviewed these older trials for their adherence to principles of methodological quality, and concluded that the available evidence does not support the use of glucocorticoids in patients with sepsis or septic shock, and that treatment may be associated with an increased mortality from secondary infections [11,12]. However, the evidence reviewed in these meta-analyses pertained exclusively to studies investigating a short course of massive doses of glucocorticoid in

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early sepsis and septic shock, and the conclusions may apply only to this restricted administration of glucocorticoid treatment.

It is likely that the positive findings of the more recent randomized studies will encourage a critical re-evaluation and stimulate additional investigation of glucocorticoids as an inexpensive and safe form of immunomodulation. When comparing the glucocorticoid trials of the past to the recent trials, three major differences are apparent: (1) timing of administration—in the recent trials, treatment was initiated 2 to 10 days after disease onset in patients that failed to improve, thus probably selecting a more uniformly sicker patient population; (2) dosage—in the past trials, the daily glucocorticoid doses employed were 5 to 140 times greater than those used in the recent trials; and (3) duration of administration—in the past trials, treatment consisted of administering one to four doses every six hours vs the strategy of the recent trials that aimed at achieving disease resolution and prolonging treatment as long as necessary (5 to 32 days).

Over the last sixty years, the focus of clinical and experimental investigation of glucocorticoid treatment in sepsis was frequently redefined by the acquisition of new medical knowledge in the fields of infectious disease, immunology, adrenal physiology, and critical care medicine. New insights into the cellular mechanisms involved in the activation of the host defense response and its regulation by glucocorticoids are important to the understanding of how changes in dosage and duration of glucocorticoid administration are associated with such disparate results [13]. This review provides an historical perspective about events that have shaped our understanding of sepsis pathophysiology and, as a result, have influenced the design of clinical trials. It concludes with a review of selected aspects of sepsis pathophysiology that are important for understanding the role of endogenous and exogenous glucocorticoids in life-threatening infections.

Design and evaluation of a treatment intervention

This section presents a structured format used in this review to evaluate clinical trials investigating glucocorticoid treatment in patients with sepsis and/or ARDS (Table 1). The purpose of this classification is to provide a guide for the design and evaluation of a treatment intervention in critically ill patients. A rationale is provided for each factor used in evaluating the relationship between disease and applied treatment. This format does not address methodological principles, that are by themselves essential but insufficient.

Terminology

Before approaching the issue of design and evaluation of a treatment intervention, however, I will briefly review basic terminology and concepts. Irrespective of its cause, a disease (dis = apart and ease = comfort)

Table 1. Factors affecting treatment response and outcome

I. Factors associated with the investigated treatment
A. Disease pathophysiological model dictating treatment design
B. Treatment directed at a central vs peripheral pathogenetic process
C. Treatment positively modifies disease pathogenesis
D. Treatment-associated complications*
E. Treatment effect is sustained until disease resolution.
II. Factors associated with the precipitating insult
A. Rapid and correct diagnosis
B. Appropriate specific treatment
III. Factors associated with the patient
A. Severity of chronic underlying illness
B. Age
C. Sex
D. Others
IV. Factors other than treatment
A. Disease severity and duration
B. Development of complications not related to treatment
C. Adequacy of support for vital organs
D. Appropriate recognition and treatment of nosocomial infections

*mask local and systemic signs of inflammation, immunosuppression, gastrointestinal bleeding, glucose intolerance

process is composed of three fundamental elements: (i) pathogenesis (mechanisms of its development), (ii) morphology (structural alterations induced in the cells and organs of the body), and (iii) functional consequences of the morphologic changes (Fig. 1) [14]. The term *syndrome* is frequently misunderstood to represent an alternative or a substitute for the term *disease*, or to imply a multifactorial etiology. *Syndrome* (syn = together and dromos = running) by definition refers to the aggregate of symptoms and signs associated with any disease [15]. In this context, *syndrome* connotes the clinical and physiological expressions (functional consequences) of the disease.

Pathophysiological models of disease

There is an unwritten understanding that the design of a therapeutic investigation rests upon two fundamental components: (1) the pathophysiological model of disease upon which treatment is tested and (2) the anticipated (biological or physiological) effect(s) of treatment on disease pathophysiology. In an upcoming article, Tolley describes the role of disease pathophysiology in the construction of a causal model investigating the cause-and-effect relationships between final outcome and events occurring during the course of a disease [16]. The construct of a disease pathophysiological model dates back to the pioneering work of Giovanni Battista Morgagni (1682–1771), a disciple of Valsalva, who first introduced the practice of correlating semeiotics (the study of symptoms and signs) with post mortem organic changes. Today, advancements in molecular biology provide an unprecedented opportu-

Fig. 1. Elements of a disease process. 1. Upregulation by transcription factors NF- κ B and AP-1; down-regulation by activated glucocorticoid receptors. 2. Cytokines TNF- α , IL-1 β , and other mediators directly transcribed by the activated transcription factors NF- κ B and AP-1. 3. The host defense response peripheral pathways—inflammation, coagulation, and tissue repair—that affect tissue morphological changes.

nity to elucidate previously unclear relationships between disease mechanisms and clinico-pathological observations.

In the construction of a disease model, pathogenesis (biology) must fit with the morphological (pathology) and clinical (physiology) findings (Fig. 1) observed during the longitudinal progress of the disease. To define need, timing, duration, and expectations of treatment, the disease pathophysiological model should delineate the differences over time between patients with a favorable (disease resolution with restoration of prior anatomy and function) and unfavorable (residual functional damage or death) course, and the time span of disease reversibility (prior to reaching end-stage disease) that is amenable to treatment. Since treatment design and the disease pathophysiological model are linked, a misunderstanding of disease pathophysiology may lead to the inappropriate application of a treatment intervention that might, otherwise, have been applied more effectively.

Treatment directed at a central vs peripheral pathogenetic process

It is reasonable to assume that the closer a treatment intervention is to the core pathogenetic mechanisms of disease development (Table 1, factor I.B), the more likely treatment will affect all “layers” of the disease

process (Fig. 1). When treatment is directed at a proximal pathogenetic event, redundancy in the host biological responses may minimize its overall impact on disease resolution. Moreover, if treatment is directed only at one or more of the criteria used to define the functional consequences of the disease (e.g., fever, tachycardia, hypoxemia, hypotension, pulmonary hypertension, etc.) without affecting its core pathogenetic process, treatment may have little impact on disease resolution.

Treatment positively modifies disease pathogenesis

The likelihood of success is increased when treatment positively modifies disease pathogenesis with an acceptable risk margin. Response to treatment—ineffective, effective, toxic—is influenced by drug dosage, interval and duration of administration. Optimal drug use depend on knowledge of both pharmacokinetics (relationship between concentration and time) and pharmacodynamics (relationship between concentration and effect) [17]. In this regard, failure to select a clear biological (or physiological) marker(s) to monitor and quantitate response to therapy may compromise estimation of true treatment effect and its impact on outcome.

Treatment effect is sustained until disease resolution

Because duration of treatment administration is an important determinant of both efficacy and toxicity, treatment duration is contingent on an understanding of disease pathophysiology and the cumulative risk associated with prolonged therapy. In determining treatment duration, consideration should be given to the time lag between a biological event and its clinical expressions, both at the onset of the disease and its resolution. For instance, if the treatment is biologically effective but of too short duration, it may be impossible to observe a clinical or physiological improvement. On the other hand, if the treatment is effective but duration is adjusted only to clinical (and not biological) resolution it may lead to premature discontinuation and disease relapse.

Additional factors affecting outcome

Finally, in measuring mortality as an end-point it is important to evaluate the influence of factors not directly related to the treatment under investigation. (Table 1). If the factors reviewed in Table 1 are not taken into consideration, even a well-designed randomized trial may not permit an appropriate interpretation of results.

Pathophysiological Model of Sepsis: Adrenal and Glucocorticoid Physiology—Part I

In the 1930s, a series of clinical and histological observations indicated that hormonal secretion by the adrenal cortex was important in the defense response against infectious and noninfectious insults, and that hormonal output was defective in nonsurvivors of sepsis (factor I.A) [18]. Prior to the introduction of cortisone, it was observed that sepsis was, in many ways, similar to an Addisonian crisis, and that patients with Addison's disease (similar to adrenalectomized animals) were hypersusceptible to infections. These patients were frequently hospitalized for upper respiratory tract infections and required large amounts of adrenal extracts to prevent Addisonian crisis and death. In 1936, Selye first described the *alarm reaction* as the initial non-specific "call to arms" of the body's defense mechanisms. Stress, generated by almost any acute threat to homeostasis, was shown to stimulate a rapid increase in hormonal production by the anterior pituitary and adrenal cortex [19]. Prior to this time, autopsy studies of soldiers dying with sepsis during World War I, and of patients dying from influenza in the early part of the century, frequently reported the presence of edema and focal necrosis of the adrenal cortex. These findings led to the assumption that inadequate adrenal function played a role in

the clinical expression and outcome of severe infections [18].

Expanding on his original work on the *alarm reaction*, Selye recognized a striking homogeneity in the systemic response of the body to acute and chronic stimuli of diverse etiology, and in an influential and comprehensive review (including over 700 references) proposed the unifying concept of *general adaptation syndrome* [19]. In this theoretical construct derived from experimental observations, much of human pathology was viewed as the by-product of the interaction between duration of exposure to an insult, and the resistance to damage offered by the hypophyseal-adrenal system [19]. Selye hypothesized that, when the stress persists beyond an acute reaction, output from the hypophyseal-adrenal system continues to offer resistance (the stage of resistance or adaptation) until a point of depletion (weeks or months from the onset), when the endocrine system response becomes inadequate and resistance breaks down (stage of exhaustion). In his view, non-specific systemic manifestations of a disease were caused by the overactivity of the adrenal cortex; the connection between local inflammation and systemic response was not established (see below). As a consequence of this incomplete understanding of cause and effect, the administration of adrenocorticotrophic hormone (ACTH) was believed to be potentially harmful and to exacerbate disease symptomatology (see relevance with rheumatoid arthritis later) [20].

Treatment of sepsis: Adrenal cortical extracts and ACTH

From 1930 to 1950, adrenal cortical extracts and ACTH were used in patients with infections as hormonal replacement (factor I.B) for presumed adrenal insufficiency, to boost host defenses, and to ameliorate symptoms of toxicity. Duration of treatment was guided by clinical observation and was continued until disease resolution (factor I.E).

In the early 1930s, a few clinical studies reported encouraging results with administration of adrenal cortical extracts in patients with typhoid fever or pyogenic sinusitis [18]. In 1939, Perla and associates reported that the administration of adrenal cortical extracts in combination with large volumes of physiologic solution was superior to either intervention alone in the prevention of experimental shock. As an extension of this work, Perla and Marmorston [18] treated seventeen patients affected with pneumonia with a combination of aggressive fluid administration (factor IV.C) and a daily intramuscular injection of adrenal cortical extracts until full clinical recovery occurred (factor I.E). This treatment modality prevented circulatory collapse, and was associated with decreased signs of toxicity, maintenance of well being, and a shorter convalescence period [18]. In 1950, Kass and associates described three seriously ill patients

with bacteremic pneumococcal pneumonia who experienced a remarkable clinical improvement with a seven day course of ACTH alone (no antibiotic administration), despite the persistence of bacteremia [21].

Pathophysiological Model of Sepsis: Adrenal and Glucocorticoid Physiology—Part II

Cortisone, an active principle of the adrenal glands, was isolated by Kendall and Reichstein in the late 1940s. In 1949, at the First Clinical ACTH Conference, Hench “electrified” the meeting with the first report on the effects of cortisone in rheumatoid arthritis [22]. The three scientists later received the Nobel Prize in Physiology and Medicine. The introduction of cortisone had important consequences on the outcome of Addison disease, the discipline of adrenal physiology, and the treatment of infections.

With adequate cortisone supplementation, patients with Addison’s disease (and adrenalectomized animals) completely lost their prior susceptibility to infections, and their infection rate became similar to that of non-Addisonian individuals. Since then, patients with Addison’s disease have been able to live nearly normal lives, while before 1939 most died within 2 years of the original diagnosis [20].

Following the introduction of cortisone, histological studies provided evidence of its striking anti-inflammatory effects at the tissue level, although the mechanisms of this pharmacological action remained elusive for years [23]. The positive impact that cortisone had on the clinical course of rheumatoid arthritis, led to investigation of its role in other fields of medicine. At the time, many clinical investigators were intrigued by the unique ability of cortisone to reduce inflammation, eliminate fever, induce a state of well-being, and alter the course of diseases of quite different etiologies. The anti-inflammatory effects of cortisone were an unpredicted and unexpected finding [20]. The striking clinical improvement observed with glucocorticoid administration contradicted part of Selye’s theory of *general adaptation syndrome* [19]. Lacking understanding of the cellular mechanisms of glucocorticoid action [13], investigators had difficulty incorporating into a unified theory two apparently opposite effects—protection during stress and suppression of inflammation, the body’s front line of defense. Instead they proposed that glucocorticoid activities could be separated into *physiological* and *pharmacological* domains [20]. To quote Munck[20]: “unable to come to terms with the antiinflammatory effects, physiologists relegated them to a pharmacological limbo, and there they have remained.” The theory that glucocorticoids provide protection during stress began to lose its status as a unifying hypothesis [20].

The introduction of cortisone provided a unique tool for investigating the role of inflammation in host-

pathogen interactions [24]. Soon most clinical studies included inflammation in their pathophysiological model of sepsis. At this time, however, inflammation was only viewed as a local tissue reaction. The systemic toxicity observed in patients with severe infections was attributed to the effects of “toxins” released from bacteria or inflammatory cells. No connection was established between the biology of local inflammation and the systemic (inflammatory) response [22]. The concept of excessive (systemic) inflammation in lethal sepsis would be introduced 15 years later [25]. Independent of its anti-inflammatory properties, cortisone was believed to protect cells from the effects of toxins (mechanism unknown).

Treatment of sepsis: Exogenous glucocorticoids

From 1950 to 1960, the pathophysiological model of sepsis comprised inflammation as a local tissue reaction to invading bacteria, systemic toxicity caused by the release of bacterial products, and presumed adrenal insufficiency in those with hypotension. Glucocorticoid treatment was used to decrease local inflammation and its sequelae, to provide cytoprotection from bacterial toxins, and to provide hormonal replacement. Duration of treatment was guided by clinical observation and was continued until disease resolution. During this period, however, two factors (Table 1) emerged that would influence glucocorticoid administration in future studies: (1) the widespread introduction of antibiotics into clinical practice (factor II) and (2) the recognition of complications associated with glucocorticoid treatment (factor I.D).

In 1950, treatment with ACTH and/or cortisone, was considered the “most notable advancement in the field of infectious disease” [26], and within two years, hundreds of experimental studies and clinical reports were published [23]. In 1950, an epidemic of streptococcal tonsillitis and pharyngitis at the Francis E. Warren Air Force base in Wyoming provided the opportunity to conduct the first randomized study involving 174 hospitalized patients [27]. A five-day course of intramuscular cortisone acetate had little effect on the clinical expression of the disease, although fewer of the treated patients developed acute rheumatic fever. In 1953, Kass and Finland [23] by reviewing the literature concluded that the administration of ACTH or cortisone to patients with acute febrile illness or with evidence of generalized toxicity frequently lead to prompt defervescence, and symptomatic improvement, while the effects of administration to patients with infections and less prominent symptoms were less striking, or even absent. By this time, the introduction of antibiotics transformed the clinical outcome of bacterial infections and made obsolete the use of glucocorticoids in patients without severe infections [28].

Exogenous glucocorticoids in combination with antibiotics

Kinsell and Jahn [22] first proposed the treatment of patients with severe infections with a combination of antibiotics (to halt bacterial proliferation) and physiological doses of glucocorticoids (to decrease the systemic effects of toxins on the body cells). In 1955, this group summarized their five-year experience in treating 300 medical and surgical patients with severe infection [22]. Their large clinical observation indicated that glucocorticoid supplementation was associated with a reduction in morbidity and mortality (see reference [29] for a critical review of this work). In 1955, Wagner reported in a randomized study of fifty-two cases of non-lethal pneumococcal pneumonia treated with penicillin, that the addition of a 5-day course of low-dose hydrocortisone (the synthetic equivalent of cortisol) resulted in clinical improvement without causing toxicity [30]. By 1957, a variety of corticosteroid regimens were evaluated in almost every type of bacterial and non-bacterial infection, and many are discussed in two important state-of-the-art reviews of the time [29,31,32]. Both reviewers concluded that there was no benefit in treating patients with mild and uncomplicated infections, and that inadequate data were available for patients with shock other than those with meningococcemia or adrenal insufficiency. For seriously ill patients, one reviewer believed that judicious and discriminate short term use in selected patients was definitively beneficial [31] while the other reviewer found the data to be promising but inconclusive [29].

Exogenous glucocorticoids and associated complications

With the wider application of cortisone in clinical practice, several centers reported that patients receiving prolonged glucocorticoid administration for the treatment of chronic inflammatory diseases or malignancies, occasionally developed life-threatening fungal and mycobacterial pneumonia and intra-abdominal infections [33]. Concern over the potential immunosuppressive action of exogenous glucocorticoids was reinforced by early experiments conducted in animals not treated with antibiotics and receiving large doses of cortisone. In these studies, large doses of cortisone induced spontaneous infections in previously uninfected animals, and compromised abscess formation, thus favoring dissemination of an otherwise localized infection [29,30,33]. The limitations of these experimental studies and the influence of glucocorticoid dosage on treatment response were recognized in many works [22,23,30,34,35].

In fact, experimental work indicated that small doses of corticosteroids may confer increased resistance to infection, although there was no clinical counterpart to this laboratory observation [29,36–38]. At the doses usually employed to treat patients, bacteriologic studies indicated no apparent impairment in antibody pro-

duction and bacterial killing [29]. The consensus among experts was that the risk of immunosuppression was low in patients receiving small doses of glucocorticoids over a short period of time, while the hazard attributable to intercurrent infection was increased in those receiving prolonged treatment [23,29,31].

Today, it is appreciated that the physiological role of glucocorticoids is not merely immunosuppressive but rather immunomodulatory. The clinical literature emphasizes the immunosuppressive role of glucocorticoids, while ignoring the fact that within a physiological concentration range they appear to both suppress and enhance immune functions [39]. The anti-inflammatory properties of glucocorticoids are integral to their role in the coordination of the immune response [39]. It has been suggested that these hormones do not simply stimulate or inhibit responses, but rather act to optimize the course of a biological response that, if delayed, would be unproductive or even dangerous to the host [40]. The complex relationship between endogenous or exogenous glucocorticoids and regulation of nonspecific, humoral, and cellular immunity is outside the scope of this chapter and the reader may refer to recent reviews of the subject [39–41].

More importantly, several reports warned that glucocorticoid administration can mask local and systemic signs of inflammation and thus impair clinical judgment in recognizing and monitoring infections (Table 1, factor I.D) [22,23]. At the time, this masking was considered the most important hazard associated with glucocorticoid treatment. To reduce the risks associated with compromising clinical evaluation, Kinsell & Jahn suggested administering glucocorticoids for the shortest time possible compatible with disease resolution (usually 5 to 7 days), with reinstitution of therapy if the patient deteriorated, and extending antibiotic therapy for five to seven days after discontinuing glucocorticoids [22].

Pathophysiological Model of Sepsis: Adrenal and Glucocorticoid Physiology—Part III

The presence of adrenal insufficiency in lethal sepsis was challenged by Melby's work on adrenal function and cortisol metabolism in experimental and clinical sepsis. In dogs injected with a lethal dose of endotoxin, they observed maximal secretion of cortisol in the circulation, and a blunted response of the adrenal glands to ACTH stimulation [42]. These animals developed a degree of systemic cellular injury proportional to the amount of injected endotoxin, with no apparent protection offered by maximal physiological output of cortisol [43]. However, when the endotoxin injection was followed by an infusion of cortisol that maintained supra-physiological plasma levels over time, the animals received protection from tissue injury and survived [43]. These investigators then conducted a prospective investigation of nine healthy adults and twenty-two pa-

tients with septic shock (most with bacteremia), and collected cortisol levels at baseline, after ACTH stimulation, and following exogenous glucocorticoid administration to determine clearance [44]. Nonsurvivors were found to have higher cortisol levels than survivors, prolonged half-life of the administered hydrocortisone, and lack of adrenal pathology at autopsy [44]. The investigators concluded that maximal cortisol secretion associated with septic shock is insufficient, and suggested that if cortisol is to be used as a therapeutic agent its action, and therefore its dosage should be pharmacological [44].

However, Kass and Finland [29] provided an alternative interpretation of Melby's data in relation to experimental work suggesting that administering low doses of cortisone improved resistance to infections [36–38]. They suggested that increased blood cortisol levels may reflect a block to steroidal activity or transport, as a consequence of the infection. In this situation, a small increase in blood levels with a low (equal or less than physiological) dose of exogenous glucocorticoids was believed to be sufficient for facilitating the passage of steroids into host cells [29]. Although both investigators agreed that maximal cortisol secretion was insufficient in septic shock, they relied on two different pathophysiological models to propose two different dosing regimens.

In the late 1950s, vascular collapse associated with Gram-negative bacilli was recognized with alarmingly increased frequency (factor IV.A). At this time, the pathogenetic association with the endotoxic lipopolysaccharide (LPS) component of the bacterial cell wall was well established, while the pathogenesis of shock was poorly understood.

Treatment of septic shock: Glucocorticoid administration in supraphysiological doses

Removing adrenal insufficiency from the pathophysiological model of lethal sepsis (factor I.A) removed the rationale for the use of glucocorticoids as physiological replacement. As experimental and clinical investigation shifted predominantly to the study of Gram-negative septic shock, a series of observations led to increasing the daily glucocorticoid dosage from physiological to supraphysiological (an approximate doubling of a physiological dose). The rationale for glucocorticoid treatment relied on the findings of animal studies showing protection in experimental endotoxemia, and an ability to potentiate the pressor response to norepinephrine in shock (factor IB) [29,31]. These activities of glucocorticoids had been recognized since 1951, but the mechanisms were unknown. In later studies, the duration of treatment was not necessarily guided by clinical observation and frequently was not reported (factor I.E).

Spink first investigated glucocorticoid administration in supraphysiological doses for the treatment of

bacterial septic shock [24]. The author reported a 10-year experience (1950–1959) involving 38 patients, 25 of whom had Gram-negative bacteremia. The work of Melby [44] and the unfavorable results observed in the early part of his experience led Spink to double the dosage of hydrocortisone to 300–500 mg every 12 hours for the first 2–3 days with rapid tapering over two days. The increased dosage was not associated with harmful effects and gave the impression of an improved response, although precise data were not provided. The overall mortality was 60%, with most deaths occurring in elderly patients with Gram-negative bacteremia. The treatment regimen (dose and duration) introduced by Spink [24] was followed with minor modifications until 1970.

Between 1950 and 1971, 32 clinical reports on glucocorticoid therapy in bacterial infections were published and were the subject of an influential review by Weitzman and Berger [45]. In these studies, the maximal daily dose was hydrocortisone 1000 mg or methylprednisolone 160 mg. Twenty-two papers considered glucocorticoids to be beneficial, 8 observed no difference, and 2 found them to be harmful [45]. Weitzman and Berger examined these studies for adherence to modern methodological standards of clinical investigation; this review became the reference point for future investigations. The standards included prospective design, concurrent controls, random allocation, double-blind procedure, stratification according to disease severity, consideration of underlying disease, and reporting of complications. Only one of the 32 studies fulfilled all criteria, and the lowest adherence was found among reports of patients with septic shock [45]. Duration of glucocorticoid administration, an important determinant of both efficacy and toxicity, was reported in only eight studies (average 7 days, range 2 to 21 days). The reviewers concluded that controversy over the role of glucocorticoids in sepsis could only be resolved if future studies would adhere to sound principles of clinical trial design.

Pathophysiological Model of Sepsis: Excessive Inflammation—Part I

Excessive inflammation and outcome

In 1965, Breen and Talukdar [25] proposed the concept that excessive (systemic) inflammation can affect outcome in sepsis. To quote: “The inflammatory reaction is a stereotyped nonspecific reaction evoked by a wide range of parasites. It is generally regarded as a defense reaction; but this must seem doubtful unless we are prepared to accept the paradox that, if the reaction be sufficiently severe, the defense may destroy the host.”

Experimental models misrepresented human sepsis

Although systemic inflammation was incorporated into the pathophysiological model of sepsis, the longitudinal

construct of the disease relied almost exclusively on experimental literature that misrepresented human sepsis. Animal experiments have usually relied on a controlled and reproducible model of sepsis produced by a short exposure to bacteria or bacterial products. Until the mid-1970s, most experiments utilized an intravenous (I.V.) bolus of lipopolysaccharide (LPS) in dogs [43,46–50] or primates [51]. This animal model, however, generates only a short dose-dependent rise in TNF [52,53], and does not reproduce the true biology of human sepsis [54]. Challenging animals with a bolus of LPS or viable bacteria reproduces an “intoxication” more than an infection [55].

From the findings of these experimental studies the notion developed that glucocorticoid anti-inflammatory effectiveness is limited to a short therapeutic window early in the course of septic shock. For example, Shumer believed that once the release of inflammatory mediators was induced through the interaction of endotoxin and complement, repeated doses of glucocorticoids would be ineffective in modulating the ensuing uncontrollable inflammatory cascade [56].

Treatment of Sepsis: Glucocorticoid Administration in Massive Doses—Part I

In 1970, a remarkable change took place in the regimen of glucocorticoid administration; this change still influences the design of clinical trials in sepsis. In experimental models, the massive and short-lived release of mediators that followed a lethal intravenous bolus of endotoxin could be offset only by the administration of equally massive doses of glucocorticoids given before or within an experimentally generated inflammatory window. As a result, the daily dosage of intravenous glucocorticoids in clinical trials increased twenty-fold or greater, while drug administration was limited to one or two boluses given a few hours apart. The fundamental concept that treatment should be directed to, and continued until disease resolution was now omitted from the design of glucocorticoid treatment investigation in sepsis (factor I.E). As the dosage escalated the risk for immunosuppression increased (factor I.D) and this concern reinforced the need for a short duration of administration. Since then, a long series of negative trials in sepsis have predominantly utilized a short course of anti-mediator treatment.

In 1970, Motsay and collaborators [49] found that dogs receiving a combination of methylprednisolone 30 mg/kg and volume replacement within five minutes of precipitating lethal endotoxic shock, preserved vascular integrity of the viscerocutaneous circulation and improved hemodynamics. A similar result had been previously obtained by Lillehei and collaborators [47]. This experimental work was followed by the first prospective, uncontrolled trial investigating a short course of massive doses of intravenous methylpred-

nisolone (30 mg/kg bolus, repeated after 2–4 hours if not effective) in fifteen patients with Gram-negative shock [57]. The protocol included volume replacement guided by hemodynamic measurements (factor IV.C) and an aggressive search for, and treatment of infections (factor II); mortality was only thirteen percent [57].

Taking into account the criticism of Weitzman and Berger [45], Shumer and collaborators published in 1976, the first prospective, randomized study evaluating massive doses of methylprednisolone. They studied 172 consecutive patients with shock and bacteremia (most Gram-negative), admitted to a surgical intensive care unit over a period of eight years [56]. Saline was compared to a single bolus of either methylprednisolone 30 mg/kg or dexamethasone 3 mg/kg, and treatment was repeated once after 4 hours, if necessary. Mortality within one month of study entry was 10.4 percent in treated patients and 38.4 percent in controls. Steroids provided protection regardless of the associated condition or etiology of sepsis.

The results of this study, however, were never reproduced, although one later study, found a survival advantage in the subgroup of patients with Gram-negative bacteremia receiving methylprednisolone [8]. The positive results obtained in the clinical studies of Motsay [57] and Shumer [56] offset initial concerns about the validity of the experimental design used to justify a short course of massive doses of glucocorticoids (see discussion following the article in reference [56]).

Treatment of Sepsis: Glucocorticoid Administration in Massive Doses—Part II

In the late 1970s, the limitations (discussed above) of this animal model were fully recognized, and the I.V. bolus administration of LPS or Gram-negative bacteria was replaced by an infusion of a few hours duration [58]. In either a canine or primate model, a lethal infusion of *E. coli* reproduced the hemodynamic, metabolic and morphological changes observed in human septic shock [58]. During this period, experimental data rapidly accumulated describing a broad range of anti-inflammatory effects associated with glucocorticoids. Glucocorticoids were shown to interfere with neutrophil function blocking the release of lysozyme and oxygen radicals, to inhibit phospholipase A₂ (PLA₂), to interfere with the activated complement cascade, and to preserve vascular integrity [59].

In the late 1970s, Hinshaw and collaborators published a series of experimental studies evaluating the response to (30 mg/kg) methylprednisolone initiated at various times from initiation of a two-to-five-hour *E. coli* infusion. These investigators found that methylprednisolone treatment did not compromise bacterial killing and that appropriate antibiotic therapy (factor

II) was essential to survival [60,61]. More importantly, these investigators recognized early in their work that the half-life of methylprednisolone is only three to four hours, and that the early beneficial response to a 30 mg/kg bolus could be sustained only if therapeutic levels were maintained with a continuous infusion of methylprednisolone (factor I.E.) [58,60,61]. With a continuous infusion, outcome was improved, even when initiation of methylprednisolone treatment was delayed by a few hours [62].

In the 1980s, five additional randomized trials were conducted investigating massive doses of glucocorticoids with a duration of administration equal to or less than 24 hours [5–9]. Contrary to Shumer's study [56], all new trials reported negative results. A few had suggested that prolonging duration of administration may have improved treatment response, but the experimental literature supporting this view (see below) was overlooked. In the last two randomized studies, the dosage of methylprednisolone was increased to 120 mg/kg over a 24-hour period, the equivalent of a one-year supply of Prednisone 20 mg tablets [7,9]. Methylprednisolone overdose was associated with a significant increase in nosocomial infection-associated mortality (factor I.D.) [7], a finding similar to that reported by McCabe and Jackson [63]. A possible explanation for this finding may be that the cytokine response to LPS challenge in humans is significantly enhanced by a prior (12 to 144 hrs before) short course of glucocorticoids [64]. A critical review of these randomized studies was provided by Sijolin [59].

Experimental evidence to support prolonged glucocorticoid treatment in sepsis and ARDS

In addition to the work of Hinshaw and collaborators [58,60,61], a significant body of experimental literature has provided clear, although unappreciated, evidence that dosage (factor I.C.) and duration (factor I.E.) of glucocorticoid administration influence treatment response and outcome in septic shock and in ARDS [46,47,65–71]. In 1956, Levitin and collaborators demonstrated that, when exogenous glucocorticoids are given by different routes of administration, protection from endotoxin correlated with blood levels and rates of disappearance from the circulation. [46]. In 1963, Lillhei showed that dogs injected with a lethal dose of endotoxin maintained aortic and renal pressure during an infusion of hydrocortisone (50 mg/kg), while the protective effects rapidly disappeared with discontinuation of administration [47]. Pitacairn found that, when male Sprague-Dawley rats were injected with a lethal dose of *E. coli*, concomitant administration of dexamethasone, with or without antibiotics, dramatically improved survival at 8 hours. However, as circulating dexamethasone levels decreased over time, the early benefits were lost and mortality increased by 20 hours [66]. In 1982, Greis-

man reported a study designed to investigate the impact that dosage and duration of administration have on methylprednisolone's efficacy in preventing murine Gram-negative mortality not preventable by antibiotics alone [67]. Outbred mice were injected intraperitoneally with one LD90-100 dose of *Escherichia coli*, *Proteus mirabilis*, or *Klebsiella pneumoniae*. Standardized models were created by selecting a time delay in initiating antibiotic therapy that resulted in 50–70% mortality. In these models, methylprednisolone was tested at 3 different concentrations, and either as single or repeated injections at 4 hour intervals. A methylprednisolone dose of 30mg/kg provided superior protection to either 10 mg/kg or 60 mg/kg, either as single or multiple injections. More importantly, they observed a significant incremental reduction in mortality with repetitive administrations [67]. Similarly, in a rat model of butylated hydroxytoluene-induced acute lung injury, glucocorticoid administration was shown to be effective in decreasing lung collagen and edema formation as long as treatment was prolonged, while withdrawal rapidly negated the positive effects of therapy [69–71].

Clinical evidence to support prolonged glucocorticoid treatment in sepsis and ARDS

Two of the five randomized studies provided useful data for evaluating the impact of treatment duration on physiological response [5,6] and outcome in patients with septic shock [6]. Lucas and Ledgerwood [5] observed a significant increase in mean arterial blood pressure during the 48 hours of dexamethasone (6 mg/kg) infusion, while this positive response disappeared when glucocorticoid therapy was discontinued. Sprung and collaborators [6] reported significant benefits early in the course of treatment. Reversal of shock, defined by strict criteria, was significantly higher in treated patients (58% vs 38%; $P < 0.05$), and the difference was more striking in those treated within 4 hours of developing shock (73% vs 20%; $P < 0.05$), or in those who survived greater than 12 hours (71% vs 14%; $P < 0.05$). Mortality 133 hours after treatment was 40% vs 69% ($P < 0.05$). The differences in reversal of shock and mortality disappeared later in the course of the study, thereby leading the authors to speculate that continuation of treatment might have sustained this initial improvement. Since the rate of elimination of methylprednisolone is significantly affected by the presence of shock (half-life 9 to 20 hours) the pharmacological effect of glucocorticoid treatment may have extended beyond 72 hours and provide an explanation for the above findings. Recent clinical work supporting prolonged administration in septic shock and unresolving ARDS is presented later (see Treatment of sepsis: rationale for prolonged glucocorticoid administration).

Treatment of Sepsis: Prolonged Glucocorticoid Administration

In the late 1980s, the negative results reported by multicenter randomized trials [5–9], the loss of financial incentive by the pharmaceutical industry resulting from expiry of patent protection, and the development of promising new anti-mediators led to a rapid decline in research on glucocorticoid treatment in sepsis and ARDS. Interest in the use of these compounds returned not as an anti-inflammatory therapy *per se* but as a modality to halt progression of histologically-proven fibroproliferation in patients with unresolving ARDS [72,73], or to provide hormonal supplementation

for presumed relative adrenal insufficiency and/or to enhance vasopressor response in patients with septic shock [74,75]. We have recently provided data indicating that pulmonary fibroproliferation in ARDS shares a common pathogenetic mechanism with other fibroproliferative diseases; in the absence of inhibitory signals, the continued production of inflammatory mediators (Fig. 2) sustains connective tissue accumulation precluding restoration of tissue structure and function (maladaptive response) [76]. We now appreciate that glucocorticoid therapeutic effects in unresolving ARDS are not limited to their anti-fibrotic activity (see later) [77]. These and many other similar reports were met with great skepticism. A verdict of “guilt by asso-

Fig. 2. Plasma inflammatory cytokines levels over time in survivors and nonsurvivors. Plasma TNF- α , IL-1 β , IL-6, and IL-8 levels from day 1 to day 10 of sepsis-induced ARDS. On day 1 of ARDS, nonsurvivors ($n = 17$) had significantly higher ($P < 0.001$) TNF- α , IL-1 β , IL-6, and IL-8 levels. Over time, nonsurvivors had persistent elevation, while survivors ($n = 17$) had a rapid decline. These findings indicate that loss of autoregulation is an early phenomenon. (Data obtained from references [101] and [90]).

ciation" (quotation from D. Schaberg) was rendered without taking into account the new pathophysiological evidence described below.

Pathophysiological Model of Sepsis: Excessive Inflammation—Part II

Sepsis is the culmination of a series of events initiated by microorganisms or their products [78]. It is now recognized that when inflammatory signals reach the cell surface, they initiate a series of events leading to activation of cytoplasmic transcription factors—DNA-binding proteins that regulate the transcription of target genes into messenger RNA. Important transcription factors associated with the host defense response include nuclear factor- κ B (NF- κ B), NF-IL-6, activator protein-1 (AP-1), the glucocorticoid receptor complex, and the heat shock transcription factor (HSF) [79].

Nuclear factor- κ B is recognized as the critical transcription factor for the maximal expression of multiple cytokines. In unstimulated cells, NF- κ B is found in cytoplasm bound to the inhibitory protein I κ B (Fig. 3). When cells are stimulated by inflammatory signals, specific kinases phosphorylate I κ B and cause its rapid degradation. The activated form of NF- κ B then rapidly moves to the nucleus, initiating the mRNA transcription of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-2, IL-6), chemokines (IL-8, etc.), cell adhesion molecules (intercellular adhesion molecule-1, E-selectin, etc.), and inflammation-associated enzymes (cyclooxygenase (COX), PLA₂, inducible nitric oxide (iNOS)) [80].

Cytokines of the interleukin-1 (IL-1) and tumor necrosis factor (TNF) families are proximal mediators of key aspects of the host defense response, including inflammation [81], coagulation [82], tissue repair [83–85], immunity [86], and activation of the hypotha-

Fig. 3. Interaction between nuclear factor- κ B and the activated glucocorticoid receptor: When cells are stimulated by inflammatory signals, specific kinases phosphorylate the inhibitory protein I κ B and causes its rapid degradation. The activated form of NF- κ B then moves to the nucleus initiating the transcription of mRNA of pro-inflammatory cytokines, chemokines, cell adhesion molecules, and inflammation-associated enzymes (cyclooxygenase, phospholipase A₂, inducible nitric oxide). Cortisol or exogenous glucocorticoids freely cross into the cytoplasm and bind to their specific glucocorticoid receptors (GR). Activated GRs inhibit the transactivational effects of NF- κ B via a dual mechanism by: (1) physically interacting with the p65 subunit; and (2) inducing the I κ B inhibitory protein, which traps NF- κ B in inactive cytoplasmic complexes catabolized by the ubiquitin-proteasome pathway [108–110]. (Modified with permission from reference [130]).

lamic pituitary adrenal (HPA)-axis (Fig. 1) [87,88]. This interactive network of simultaneously activated pathways work in synergy to eliminate or control the noxious insult and to repair any consequent tissue damage [89]. However, if unchecked and unregulated, a protracted and exaggerated release of "inflammatory" mediators leads to deterioration in organ function rather than restoration of homeostasis [90–92].

Increased activation of NF- κ B has been demonstrated in the peripheral monocytes of patients with sepsis and in the alveolar macrophages of patients with established ARDS [93–95]. During an observation period of 14 days, nonsurvivors of sepsis had significantly higher NF- κ B activity ($p < 0.001$) in their peripheral monocuclear cells than did survivors [95]. In agreement, several studies have reported that nonsurvivors of sepsis [54,96–101] and ARDS [101–106] exhibit exaggerated and protracted elevation of circulating proinflammatory cytokine levels. In patients with sepsis-induced ARDS we found [101] that elevated proinflammatory cytokine levels on day one predicted a persistent elevation over time (Fig. 2), suggesting that loss of autoregulation is an early event in the course of lethal sepsis.

Pathophysiological Model of Sepsis: Adrenal and Glucocorticoid Physiology—Part IV

Glucocorticoid receptors

In the late 1960s, the discovery of glucocorticoid receptors (GRs) led to the gradual realization that a single, basic molecular mechanism initiates most glucocorticoid actions [20]. Elucidation of this mechanism had a unifying influence on glucocorticoid endocrinology. In 1984, Munck, Guyre, and Holbrook published a landmark review that restored the lost concept of glucocorticoid-induced host protection during stress [20]. These authors proposed that, the physiologic function of stress-induced increases in glucocorticoid levels is protecting against the host normal defense reactions that are activated by stress, but not protecting against the source of stress *per se*. They also proposed that glucocorticoids accomplish this function by turning off those normal defense reactions, thus preventing them from overshooting and ultimately threatening homeostasis [20].

Glucocorticoids exert most of their effects through specific, ubiquitously distributed intracellular (3,000 to 100,000 per cell) GRs. GR activation and translocation has been studied extensively, and the molecular mechanisms involved in these processes were recently reviewed [79,107]. Since the GR ultimately controls pertinent gene expression, anything that affects its binding affinity, transportation to the nucleus, number, conformation or possible interaction with other relevant transcription factors can affect the response of the cells to glucocorticoids and, from the clinical point of

view, the response of the individual to glucocorticoid administration during intense inflammation.

Glucocorticoid receptors and transcription factor NF- κ B

Many of the anti-inflammatory activities of glucocorticoids are exerted by GR-mediated blockade of the activity of transcription factors NF- κ B and AP-1. Activated GRs inhibit the transactivational effects of NF- κ B via a dual mechanism by: (1) physically interacting with the p65 subunit; and (2) inducing the I κ B inhibitory protein, which traps NF- κ B in inactive cytoplasmic complexes catabolized by the ubiquitin-proteasome pathway (Fig. 3) [108–110]. By these mechanisms, activated GRs inhibit the transcription of several cytokines [107,111], inflammation-associated enzymes [107,112], and adhesion molecules (Table 2) [107]. Glucocorticoids also have an inhibitory effect on fibrogenesis [113] and act in synergy with interleukin (IL)-1 receptor antagonist [114] and the anti-inflammatory cytokines IL-4, IL-10, and IL-13 [115]. Finally, glucocorticoids influence several key regulatory systems important in the maintenance of arterial blood pressure [116].

Excessive inflammation and glucocorticoid resistance

Nonsurvivors of septic shock, contrary to survivors, have exaggerated and persistent elevation of circulating inflammatory cytokine levels [54,96–101], as well as marked elevations in plasma ACTH and cortisol levels over time [42,44,117,118]. The elevation of glucocorticoid secretion in nonsurvivors is inadequate to meet the needs of the concurrent host defense response and its adverse systemic effects. This could be

Table 2. Effect of glucocorticoids on gene transcription

Increased Transcription
Lipocortin-1
β 2-Adrenoceptor
Endonucleases
Secretory leukocyte protease inhibitor
inhibitory protein I κ B
Decreased Transcription
Cytokines (TNF- α , IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-11, IL-12, IL-13, GM-CSF, RANTES, MIP-1 α)
Inducible nitric oxide synthase (iNOS)
Inducible cyclo-oxygenase (COX-2)
Inducible phospholipase A ₂ (cPLA ₂)
Endothelin-1
NK1-receptors
Adhesion molecules (ICAM-1, E selectin)

TNF = tumor necrosis factor; IL = interleukin; GM-CSF = granulocyte-macrophage colony stimulating factor; RANTES = Regulated upon Activation, Normal T-cell Expressed and Secreted; MIP-1 α = macrophage inflammatory protein-1 α . (Reproduced with permission from reference [130]).

due to tissue resistance to glucocorticoids and/or inadequacy of the level and duration of endogenous glucocorticoid elevation to suppress a host defense response gone awry [13].

Recently, two groups of investigators have recognize that cytokines can cause a concentration-dependent resistance to glucocorticoids by reducing GR binding affinity for cortisol [119,120]. Although the exact mechanisms have not been fully clarified, researchers have postulated that an excess of cytokine-induced transcription factors, such as NF- κ B and AP-1, may form complexes with activated GRs, preventing GR interaction with DNA and the expression of glucocorticoid responsive element (GRE)-mediated anti-inflammatory activity [108]. Such inhibition of cortisol binding to GR was demonstrated in T cells incubated with a combination of IL-2 and IL-4 [120], IL-1, IL-6, and interferon (IFN)- γ [119], or IL 13 [121]. Glucocorticoid resistance was induced in a cytokine concentration-dependent fashion and was reversed by the removal of cytokines [120].

This line of evidence is reinforced by the results of experimental studies [122–124]. In a sheep model of sepsis-induced ARDS, maximal binding capacity of GR decreased continuously after endotoxin infusion, while there was a marked elevation of cortisol levels [122]. The reduced GR binding correlated negatively ($r = -0.87$, $p < 0.01$) with PLA₂ (the rate-limiting enzyme in eicosanoid metabolism) activity. In a rat model of septic shock, GR blockade by mifepristone (RU486) exacerbated the physiological and pathological changes induced by endotoxemia [123]. PLA₂ activity in rats with 80% GR blockade was more marked than in those with 50% GR blockade [123].

Monocytes of patients with sepsis develop near total glucocorticoid resistance *in vitro* when cytokines, especially IL-2 are added [125]. The reduction in glucocorticoid responsiveness, therefore, appears to become greater as the intensity of the inflammatory response increases. In addition, this inflammatory cytokine-induced glucocorticoid resistance could be increased by stimulation of the natural non-glucocorticoid binding isoform of the GR (GR β , an isoform that has dominant negative effects on the classic GR (GR α) [108,126].

Treatment of Sepsis: Rationale for Prolonged Glucocorticoid Administration

Finally, the potential usefulness of prolonged glucocorticoid treatment in patients with septic shock and unresolving ARDS is discussed in reference to those factors (Table 1, factors I.A to I.E) previously reviewed in the section on the design and evaluation of a treatment intervention. If the proposed pathophysiological construct is correct, then prolonged glucocorticoid therapy may be useful, not as an anti-inflammatory treatment *per se*, but as an hormonal supplementation to

compensate for the host inability to produce appropriately elevated levels of cortisol or for the inability of target organs to respond to endogenous cortisol (factor I.A). Adequate hormonal supplementation should modify the intracellular balance among activated transcription factors (e.g., NF- κ B, AP-1, and GR), thus converting an initially dysregulated (maladaptive) host defense response into a regulated (adaptive) one (factor I.C). In this context, glucocorticoid treatment is directed at the core pathogenetic mechanism (factor I.B) of sepsis and should affect all layers of the disease process (Fig. 1). GR-mediated blockade of NF- κ B transcriptional activity (Fig. 3) should decrease (not suppress) the synthesis of proximal and distal mediators of the host defense response, and if this beneficial response is sustained over time (factor I.E) patients should experience an improvement in physiological and clinical abnormalities (Fig. 1). Alternative modalities to block NF- κ B are being investigated [127].

For patients with sepsis or ARDS, there is no direct evidence demonstrating that glucocorticoid treatment induces a reduction in intracellular activation of transcription factors NF- κ B or AP-1. Indirect evidence for such action, however, is provided by clinical studies that have monitored, before and during prolonged glucocorticoid administration, blood concentration of several mediators transcribed by NF- κ B (Fig. 3). In these studies, treatment was associated with a rapid and significant reduction in plasma levels of inflammatory mediators (TNF- α , IL-1 β , IL-6, IL-8, PLA₂) [74,128], adhesion molecules [77], and markers of collagen synthesis [76]. As long as treatment was continued, the concentration of these biological markers continued to decrease, while physiological and clinical variables improved in parallel (factors I.B and I.C). In a randomized study of patients with sepsis-induced unresolving ARDS [4], this beneficial biological and physiological response was observed only in patients randomized to prolonged methylprednisolone administration, while no change was observed in those receiving placebo [76,77]. In three uncontrolled studies the premature discontinuation of prolonged glucocorticoid administration in septic shock [74] and in unresolving ARDS [72,129] was associated with physiological [72,74,129] and laboratory (PLA₂ and C-reactive protein levels) [74] deterioration that improved with reinstitution of treatment (factor I.E). Finally, two reports provided indirect evidence to support the concept of endogenous glucocorticoid resistance in patients with sepsis or unresolving ARDS. In a study of patients with septic shock, plasma cortisol levels decreased during hydrocortisone infusion and returned toward pre-treatment values after discontinuing therapy [74]. Similarly, in patients with unresolving ARDS we have found that prolonged glucocorticoid administration was associated with a significant reduction in ACTH and cortisol levels, in parallel with a reduction in circulating inflammatory cytokine levels (personal unpublished data).

Since the 1950s [22,23], it has been appreciated that

the most serious threat to the recovery of patients receiving prolonged glucocorticoid treatment is failure to recognize infections in the presence of a blunted systemic and local inflammatory response (factor I.D). We have previously shown that during prolonged methylprednisolone treatment of unresolving ARDS, infections frequently developed in the absence of fever; and infection surveillance, including weekly bilateral bronchoalveolar lavage is required for early detection of pneumonia and other serious infections [73]. By incorporating this surveillance program into a recently completed randomized study [4], ventilator-associated pneumonia was frequently detected and treated in the absence of fever. In this randomized study, the infection rate was not significantly affected by methylprednisolone treatment and none of the nosocomial infections with potentially lethal pathogens developing during methylprednisolone therapy affected resolution of ARDS or clinical outcome.

A recent work from our group has provided experimental evidence that exaggerated inflammation favors bacterial growth. We have reported that three bacterial species—*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp.—showed a concentration-dependent growth enhancement when incubated with graded concentrations of biologically active TNF- α , IL-1 β , and IL-6, and that blockade by specific neutralizing monoclonal antibodies significantly inhibited cytokine-induced growth [131,132]. If the clinical relevance of these findings can be proven, it is reasonable to assume that treatment modalities directed at controlling exaggerated inflammation might be useful in curtailing bacterial infections, a true paradigm shift.

Conclusions

From the early 1940s to the late 1980s, evolution in the pathophysiological understanding of sepsis and ARDS has strongly influenced the design of protocols investigating glucocorticoid treatment in this patient population. During this period of time, the daily glucocorticoid dose escalated up to 140 fold, while the duration of treatment decreased to equal or less than 24-hour. Recent evidence indicates that, in patients with septic shock or ARDS, excessive activation of the host defense response may induce noncompensated glucocorticoid resistance in target organs, a condition potentially responsive to exogenous glucocorticoid supplementation. Patients with a dysregulated host defense response have persistent immune cell activation of NF- κ B (a critical transcription factor for the maximal expression of multiple cytokines) and protracted elevation of inflammatory cytokine levels over time. In both uncontrolled and controlled studies, prolonged glucocorticoid treatment of patients with septic shock or unresolving ARDS was associated with a rapid, significant, and sustained reduction in circulating levels of these markers of disease activity, and

progressive physiological improvement. No other treatment intervention in sepsis or ARDS has yet provided this level of evidence. Although, the results of four recent randomized studies are encouraging, pharmacokinetic and pharmacodynamic studies of exogenous glucocorticoids in sepsis and ARDS are not yet available to guide optimal drug administration, and today's treatment dosing is essentially empirical. Additional research is necessary to advance our understanding of the complex mechanisms that influence endogenous and exogenous glucocorticoid activities at cellular level during acute life-threatening inflammation.

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