BRIEF REPORT

Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412

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SUMMARY

Six healthy young male volunteers at a contract research organization were enrolled in the first phase 1 clinical trial of TGN1412, a novel superagonist anti-CD28 monoclonal antibody that directly stimulates T cells. Within 90 minutes after receiving a single intravenous dose of the drug, all six volunteers had a systemic inflammatory response characterized by a rapid induction of proinflammatory cytokines and accompanied by headache, myalgias, nausea, diarrhea, erythema, vasodilatation, and hypotension. Within 12 to 16 hours after infusion, they became critically ill, with pulmonary infiltrates and lung injury, renal failure, and disseminated intravascular coagulation. Severe and unexpected depletion of lymphocytes and monocytes occurred within 24 hours after infusion. All six patients were transferred to the care of the authors at an intensive care unit at a public hospital, where they received intensive cardiopulmonary support (including dialysis), high-dose methylprednisolone, and an anti–interleukin-2 receptor antagonist antibody. Prolonged cardiovascular shock and acute respiratory distress syndrome developed in two patients, who required intensive organ support for 8 and 16 days. Despite evidence of the multiple cytokine-release syndrome, all six patients survived. Documentation of the clinical course occurring over the 30 days after infusion offers insight into the systemic inflammatory response syndrome in the absence of contaminating pathogens, endotoxin, or underlying disease.

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On March 13, 2006, eight healthy male volunteers participated in a double-blind, randomized, placebo-controlled phase 1 study of the safety of TGN1412 (TeGenero), a novel monoclonal antibody. The study drug is a recombinantly expressed, humanized superagonist anti-CD28 monoclonal antibody of the IgG4κ subclass that stimulates and expands T cells independently of the ligation of the T-cell receptor.[1] In contrast to other antibodies in clinical use or in clinical trials, TGN1412 directly stimulates the immune response in vivo. In preclinical models, the stimulation of CD28 with TGN1412 (or with murine-antibody counterparts) preferentially activated and expanded type 2 helper T cells[2] and, in particular, CD4+CD25+ regulatory T cells, resulting in transient lymphocytosis with no detectable toxic or proinflammatory effects.[1-4]

On the day of the trial, six of the eight volunteers received TGN1412 and two received placebo. Subsequently, the six volunteers in the treatment group, who had multiorgan failure with an unknown mechanism and an unpredictable severity, were all admitted to the on-site critical care unit at Northwick Park and

St. Mark's Hospital, a National Health Service (NHS) hospital in London. We detail the clinical and pathological findings during the first 30 days after the infusion.

METHODS

TRIAL CONDUCT

TeGenero sponsored the trial of the monoclonal antibody TGN1412, which was manufactured by Boehringer Ingelheim. The trial was conducted by Parexel International, a contract research organization that operates an independent clinical trials unit in leased space on the premises of Northwick Park and St. Mark's Hospital.

The authors of this report are a group of NHS clinicians who assumed clinical responsibility for the secondary care of these patients after they were transferred to the NHS (between 12 hours [one patient] and 16 hours [five patients] after infusion). The authors have no contractual or operational relationship with either Parexel International or TeGenero.

PATIENTS AND SOURCES OF DATA

All six patients provided written informed consent to the NHS for the publication of data obtained during clinical case management. Clinical data obtained before admission to the NHS, and selected laboratory data obtained before the complications were observed, are reproduced here with permission from TeGenero. The trial was suspended owing to the serious adverse events, and no further tests were performed for research purposes. There was full disclosure of drug information, scientific data, and trial documentation by TeGenero and Parexel International, in order to assist in clinical management decisions at the time of the incident.

CYTOKINE AND CELL SUBGROUP DETERMINATIONS

Data on subgroups of cytokines and lymphocytes were subsequently collected for clinical purposes during the course of the illnesses. For details on the cytokine assays and the cell subgroups, see the Supplementary Appendix (available with the full text of this article at www.nejm.org).

RESULTS

All six patients who received the trial drug were male, with a median age of 29.5 years (range, 19 to 34) (Table 1). None had a notable medical history, and all were clinically well during the 2 weeks before the study; baseline laboratory values were normal (Table 2). Beginning at 8 a.m. on day 1, each volunteer received an intravenous infusion, 10 minutes apart, of either the study drug or placebo. Each infusion lasted 3 to 6 minutes. The six volunteers in the treatment group each received 0.1 mg of TGN1412 per kilogram of body weight, infused at a rate of 2 mg per minute; the remaining two volunteers received a similar volume of saline.

INITIAL RESPONSE AFTER INFUSION OF TGN1412

A series of adverse effects began in the treatment group after infusion, starting with the onset of severe headache in five patients after a median of 60 minutes (range, 50 to 90), accompanied by lumbar myalgia in all six patients after a median of 77 minutes (range, 57 to 95) (Fig. 1). Subsequently, during this early phase, the patients were restless and had varying degrees of nausea, vomiting, bowel urgency, or diarrhea (Table 1). Five subjects had short amnestic episodes associated with severe pyrexia, restlessness, or both. All patients had a systemic inflammatory response that included erythema and peripheral vasodilatation (the timing of which was undocumented), with recorded rigors in four patients at a median of 59 minutes (range, 58 to 120) after infusion. Hypotension (defined by a decline in systolic blood pressure of 20 mm Hg or more) developed in all patients a median of 240 minutes (range, 210 to 280) after infusion, accompanied by tachycardia, with maximal heart rates of 110 to 145 beats per minute. All patients received intravenous lactated Ringer's solution during this time. Body temperatures of 39.5 to 40.0°C were recorded a median of 280 minutes (range, 240 to 390) after infusion. At 300 minutes after infusion, Patient 1 had signs of respiratory failure, with tachypnea and a partial pressure of arterial oxygen (PaO_2) of 52 mm Hg while breathing ambient air; the PaO_2 increased with the addition of supplemental oxygen. Chest radiography revealed pulmonary infiltrates; these findings were not consistent with the expected response of a fit young man to the infusion of less than 4 liters of fluid at this stage. There was no clinical evidence of bronchospasm or laryngeal edema.

All patients were initially empirically treated

Table 1. Data for All Six Affected Patients on Transfer to the Intensive Care Unit (ICU).*

Characteristic	Patient No.					
	1	2	3	4	5	6
Age (yr)	24	34	31	19	28	20
Weight (kg)	68.9	84.3	81.8	72.1	88.5	82.4
TGN1412 dose (mg)	6.8	8.4	8.2	7.2	8.8	8.2
Transfer to critical care (hr after dose)	15.5	16.0	16.0	16.0	16.0	12.0
APACHE II score on transfer†	8	10	11	18	20	18
Bilateral pulmonary infiltrates‡	+	++	++	++	++	+++
Duration of abnormalities on chest radiography (days)	7	6	8	>5	6	7
Hemodynamics on transfer						
Blood pressure (mm Hg)	120/50	124/79	107/42	98/40	95/40	80/64
Heart rate (beats/min)	125	103	116	120	105	140
LVEF on echocardiogram (%)	50–55	70	60	50–55	60	55
PaO_2:FIO_2	395.5	195.6	329.5	321.3	201.8§	84.0§
Base deficit (mmol/liter)	–5.1	–6.5	–5.6	–5.8	–10.3	–8.2
Lactate (mmol/liter)¶	3.1	4.5	5.7	6.0	5.9	4.2
Urinary output (ml/hr)	20	30	30	45	30	0
Treatment						
Days spent in ICU	4	7	7	5	11	21
Days receiving corticosteroids (including tapering)	21	21	21	21	24	33
Epiphenomena						
Generalized desquamation‖	+	++	+	+	++++	+++
Muscle weakness‡	+	++	+	+	++	++
Late myalgia	Calf	Calf and hip adductors	Calf	—	Calf	—
Neurologic findings	Headaches and hyperalgesia	Hyperalgesia	Hyperalgesia and numbness	Headaches	Headaches and numbness	—

* LVEF denotes left ventricular ejection fraction, PaO_2 partial pressure of arterial oxygen, and FIO_2 fraction of inspired oxygen.
† Acute Physiology and Chronic Health Evaluation (APACHE) II scores range from 0 to 71, with higher values indicating more severe illness.
‡ Plus signs represent the degree of infiltrates or of muscle weakness.
§ The patient was on assisted mechanical ventilation.
¶ The normal range for lactate is 0.5 to 2.2 mmol per liter.
‖ One plus symbol represents one episode of generalized desquamation, two represent two episodes, and three and four represent increasingly prolonged generalized desquamation.

in the independent clinical trials unit. A dose of 200 mg of hydrocortisone was administered intravenously in divided doses (with the initial 100-mg bolus a median of 331 minutes [range, 315 to 346] after infusion), in addition to 10 mg of chlorpheniramine intravenously, 1 g of acetaminophen intravenously, 4 to 8 mg of ondansetron intravenously, and 0.5 to 3.0 mg of metaraminol intravenously (in divided doses, titrated to effect). Blood samples were analyzed 8 hours after infusion at an off-site private laboratory (according to the study protocol) and therefore were not available as the situation evolved; the results were abnormal (Table 2).

SUBSEQUENT EVENTS

After an initial recovery, Patient 6 became hypotensive (blood pressure, 65/40 mm Hg), and 12 hours after infusion, he had metabolic acidosis and marked respiratory distress with hypoxemia that was refractory to treatment with supplemental oxygen. He underwent intubation and mechanical ventilation, after which he was admitted to the intensive care unit (ICU) at Northwick Park

Table 2. Median Results of Blood Tests for the Six Patients before Infusion and 8 and 16 Hours after Infusion of TGN1412.*

Blood Level of Constituent	Independent Clinical Trials Unit			Intensive Care Unit	
	Before Infusion	8 Hours after Infusion	Normal Range	16 Hours after Infusion	Normal Range
Creatinine (μmol/liter)					
Median	80	128	—	163	—
Range	74–89	106–195	66–112	125–325	62–115
Urea (mmol/liter)					
Median	4.8	6.4	—	9.3	—
Range	3.6–6.0	6.1–7.6	1.7–8.3	7.3–7.7	3.2–7.4
Uric acid (μmol/liter)					
Median	330	418.5	—	404	—
Range	309–426	339–465	266–474	251–590	210–420
Alanine aminotransferase (IU/liter)					
Median	25	21	—	32	—
Range	22–36	15–191	10–50	18–161	0–55
Hemoglobin (g/dl)					
Median	15.4	11.7	—	12.7	—
Range	15.1–15.9	11.0–14.1	13.0–17.0	10.3–15.6	13.0–17.5
Neutrophils ($\times 10^{-3}$/mm^3)					
Median	2.43	2.31	—	6.50	—
Range	1.73–5.14	1.99–4.95	2.00–7.50	5.09–11.14	1.80–7.70
Monocytes ($\times 10^{-3}$/mm^3)					
Median	0.26	0.03	—	0.03	—
Range	0.08–0.59	0.01–0.15	0.20–1.00	0.01–0.05	0.20–0.80
Lymphocytes ($\times 10^{-3}$/mm^3)					
Median	1.86	0.06	—	0.04	—
Range	1.47–2.59	0.05–0.09	1.50–4.00	0.03–0.07	1.10–4.80
Platelets ($\times 10^{-3}$/mm^3)					
Median	222	98	—	132	—
Range	164–261	51–144	150–400	69–169	140–450
Prothrombin time (sec)					
Median	11.2	14.2	—	26.2	—
Range	10.5–11.7	13.1–19.5	10.0–12.0	19.5–33.2	11.5–16.0
Activated partial-thromboplastin time (sec)					
Median	NA	NA	—	43.5	—
Range				40.1–61.9	26.0–38.0
Fibrinogen (g/liter)					
Median	NA	1.47	—	1.69	—
Range		0.66–1.75	1.50–4.00	0.99–1.98	2.00–4.50
D-dimer (ng/ml)					
Median	NA	NA	—	1784	—
Range				1350–4535	0–250

* To convert values for creatinine to milligrams per deciliter, divide by 88.4. To convert values for urea to milligrams per deciliter, divide by 0.357. NA denotes not available.

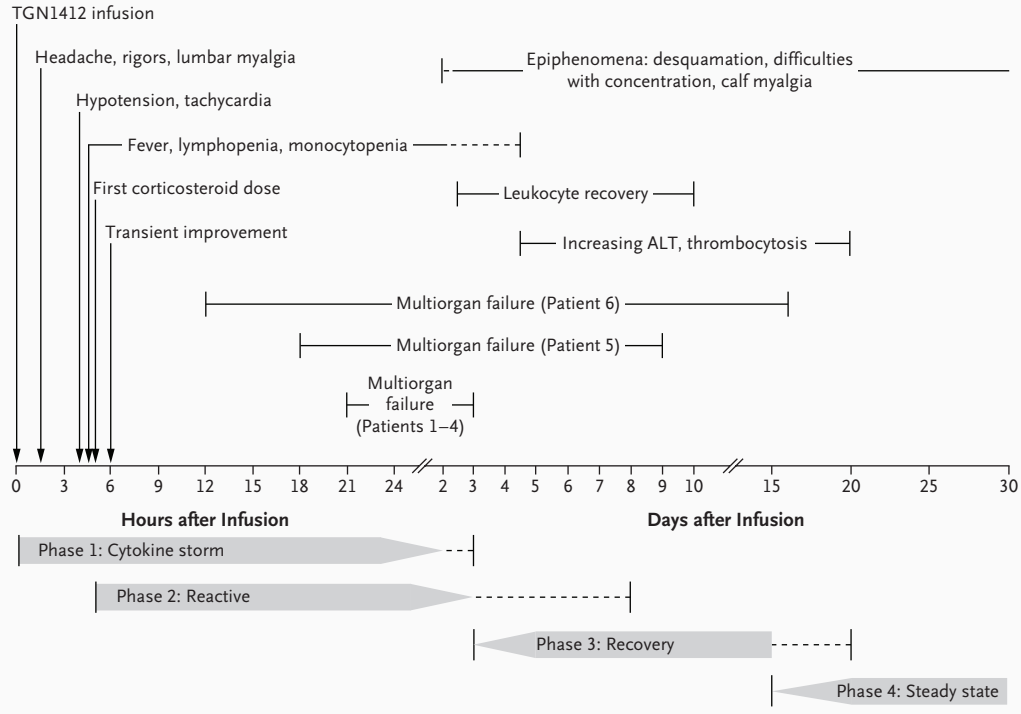


Figure 1. Summary Timeline of the Main Events after Infusion of TGN1412.

The course is divided into four phases: cytokine storm, reactive, recovery, and steady state. ALT denotes alanine aminotransferase. Dashed lines represent the responses of Patients 5 and 6 (who were the most seriously ill).

and St. Mark's Hospital. He had severely abnormal hemodynamics, coagulation, and pulmonary function, with a PaO_2 of 84 mm Hg while breathing 100% oxygen (ratio of PaO_2 to the fraction of inspired oxygen, 84) (Table 1).[5] Because there was concern that all patients would follow a similar course of rapid deterioration, all remaining patients were transferred to NHS ICU facilities 16 hours after infusion.

FURTHER TREATMENT

Between 16 and 20 hours after infusion of TGN1412, the patients had further signs of respiratory deterioration: all six had signs of tachypnea, use of accessory muscles, inability to complete spoken sentences, and bilateral pulmonary infiltrates on chest radiography (Fig. 2A and 2B), and two had symptoms of dyspnea. There was also evidence of substantial renal impairment and disseminated intravascular coagulation, as indicated by an elevated prothrombin time, low fibrinogen level, high level of D-dimers, and decreased platelet counts in all six patients (Table 2). All patients had severe lymphopenia and monocytopenia, with sparing of neutrophils. Blood smears showed toxic granulation with Döhle's bodies and a dysplastic appearance of the neutrophils, with pseudo–Pelger–Huët anomaly (Fig. 2C and 2D).

There was no clinical evidence of primary cardiogenic shock, nor was there bronchospasm, laryngeal edema, or cutaneous signs indicating anaphylaxis. There were no overt or focal neurologic symptoms or signs that suggested neurogenic vasodilatory shock. All electrocardiograms and echocardiograms were normal (Table 1), and there was no clinical indication for lumbar puncture or electroencephalography.

All patients received empirical treatment with 1 g of methylprednisolone sodium succinate intravenously a median of 16 hours (range, 15.5 to 17) after infusion with TGN1412, with subsequent doses 40 hours and 64 hours after. Because of the expected effects of TGN1412 on T cells, all patients were empirically treated daily for 3 days with an anti–interleukin-2 receptor antagonist antibody, daclizumab (Roche), beginning a median of 25.5 hours (range, 23.5 to 28.0) after infusion. This treatment was stopped after 3 days

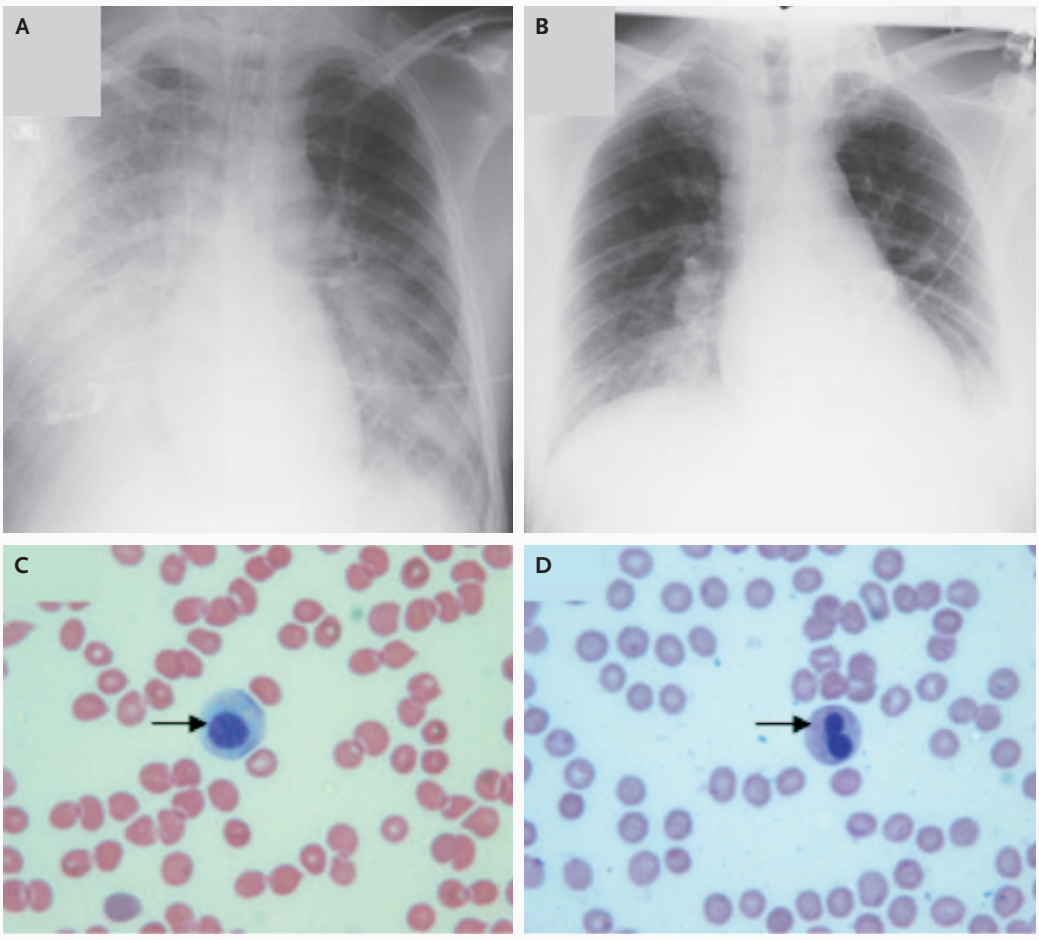


Figure 2. Representative Chest Radiographs (Panels A and B) and Blood Smears (Panels C and D) of the Six Affected Patients.

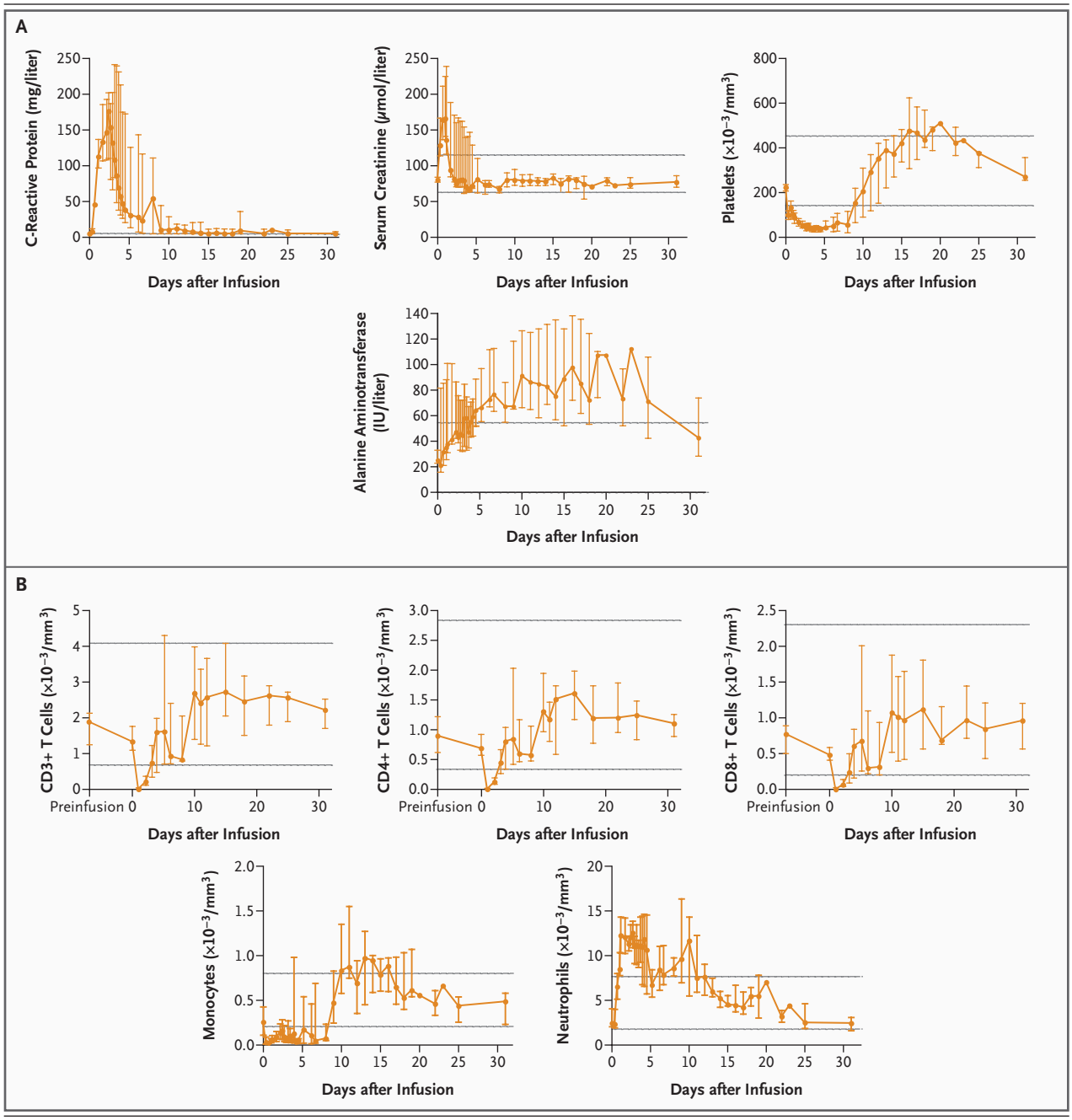
All anteroposterior chest radiographs were similar in appearance, with interstitial infiltrates first noted 5 to 16 hours after infusion of TGN1412 (Panels A and B). The blood films did not show red-cell fragmentation but did show dysplastic changes in the neutrophils, including pseudo–Pelger–Huët anomaly (arrows, Panels C and D; May–Grünwald–Giemsa stain), which was first noted within 24 hours after TGN1412 infusion. Although toxic granulation, vacuoles, and Döhle's bodies were initially observed, later blood smears showed neutrophils that were hypogranular.

in the absence of TGN1412-induced lymphocytosis. In addition, potential activation of a histaminergic response was treated with 50 mg of intravenous ranitidine every 8 hours and 10 mg of intravenous chlorpheniramine maleate every 8 hours (continued from earlier doses).

SUPPORTIVE MANAGEMENT

Patients 1 through 4 received continuous positive airway pressure of 10 cm H_2O by means of a tight-fitting face mask. Patients 5 and 6 underwent mechanical ventilation, with tidal volumes limited to 6 to 8 ml per kilogram of dry body weight and positive end-expiratory pressure maintained at 15 to 20 cm H_2O. All six patients had oliguria, metabolic acidosis, and increasing creatinine levels; they therefore received renal support by means of continuous venovenous hemodiafiltration with the use of a standard polyacrylonitrile membrane (Gambro Hospal U.K.) within 36 hours after their exposure to TGN1412. Dialysate rates were set to 1 liter per hour and were subsequently increased to 4 liters per hour.

All patients required the replacement of blood components by means of the infusion of fresh-frozen plasma and cryoprecipitate to correct co-



agulopathy. Owing to their severe lymphopenia, the patients were treated according to a protocol of infusions of irradiated red cells and platelets, as required, to prevent possible graft-versus-host disease.

CLINICAL PROGRESSION

Patients 1, 2, 3, and 4 continued to have intermittent fever, myalgia, and diffuse erythematous flushing for 48 hours, at which point their clinical symptoms and signs diminished markedly. Immunomodulatory treatment in these four patients was reduced to a tapering dose of intravenous hydrocortisone followed by oral prednisolone (total duration of corticosteroid treatment in each case, 21 days). Continuous venovenous hemodiafiltration was stopped after a median of 28 hours (range, 22 to 35), and continuous positive airway pressure was stopped after 4 hours in Patient 1 and after a median of 77 hours (range, 57 to 82)

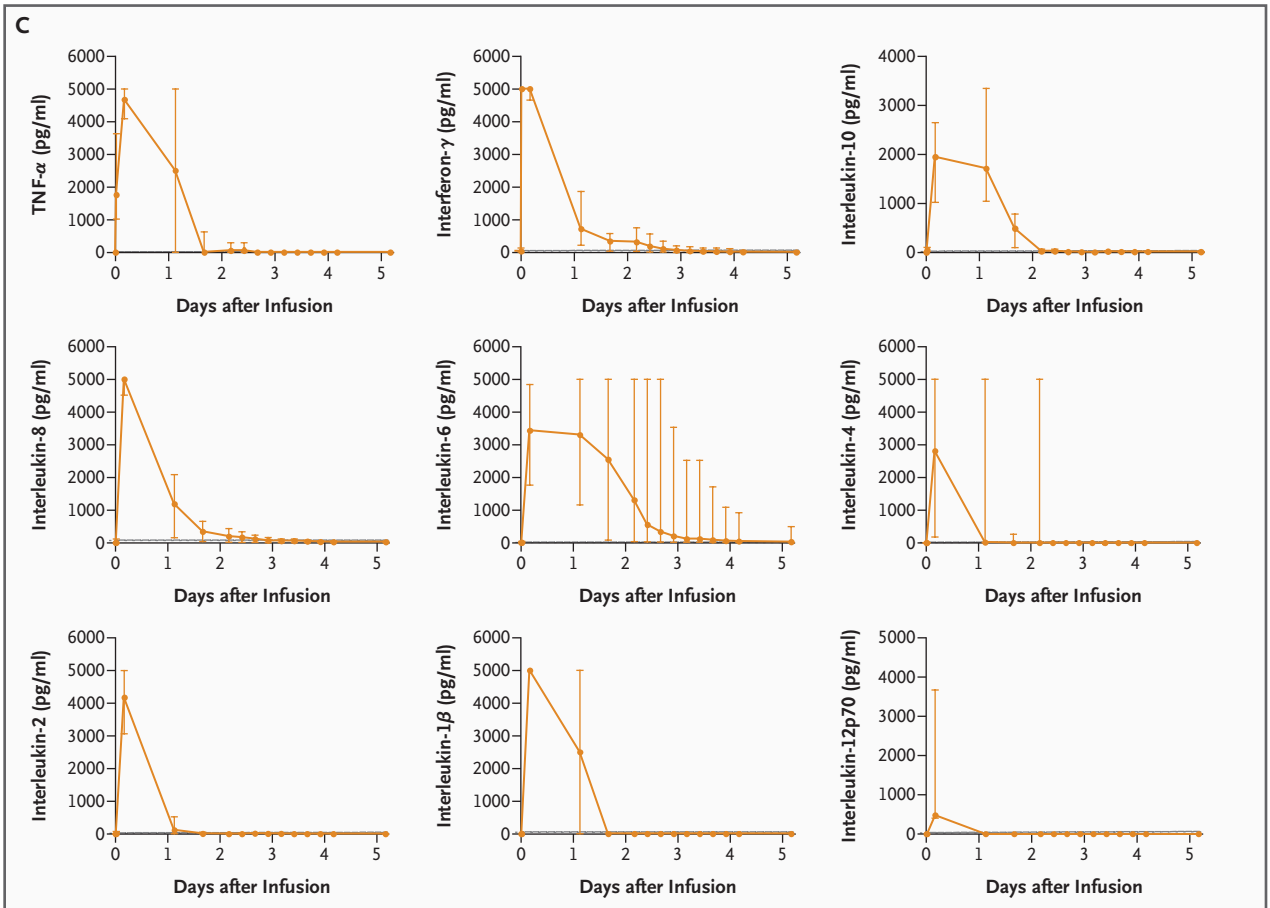


Figure 3. Summary of Laboratory Results for the Six Patients during the First 30 Days (Panels A and B) and the First 5 Days (Panel C) after Infusion of TGN1412.

Panel A shows that C-reactive protein and serum creatinine levels increased rapidly during the first 48 hours after infusion, with a concomitant decline in the platelet count starting within the first 8 hours and persisting for at least 5 days. Alanine aminotransferase levels increased slowly, starting within the first 48 hours, and peaked between 10 and 25 days after infusion, when the patients had recovered from the acute illness. Panel B shows that levels of CD3+, CD4+, and CD8+ T-cell subgroups were undetectable within the first 24 hours after infusion, followed by a first peak at day 5 and a second peak at day 15, with a leveling off to near-preinfusion levels by day 30. Monocyte numbers also fell in the short term but increased to above the normal range 10 to 16 days after infusion. Neutrophil counts were relatively constant immediately after infusion and then increased, as expected, with increasing stress and corticosteroid use. Panel C shows that, during the first 4 hours after infusion, the first cytokine to increase substantially was TNF-α (2.8 pg per milliliter at 0 hour, 1760.1 at 1 hour, and 4675.9 at 4 hours), followed by interferon-γ (7.1 pg per milliliter at 0 hour, 43.9 at 1 hour, and ≥5000 at 4 hours) and interleukin-10, 8, 6, 4, 2, 1β, and 12p70. All data are medians. I bars represent interquartile ranges. Dashed lines represent the upper limit of the normal reference range (where only one dashed line is shown) or both the upper and lower limits. Time points with single values were excluded. To convert values for creatinine to milligrams per deciliter, divide by 88.4.

in Patients 2, 3, and 4 (Fig. 4A and 4B through 7A and 7B in the Supplementary Appendix). Patient 2 was also successfully treated for presumed nosocomial *Klebsiella pneumoniae* bacteremia, isolated on day 6 after TGN1412 infusion.

Patients 5 and 6 had a more complex course, as detailed in the Supplementary Appendix. Although both patients initially had diminished erythema and fever 48 hours after infusion, they subsequently had recurrent fever, increased peripheral vascular permeability, and episodes of diffuse erythematous flushing lasting several days. Both patients required intubation and mechanical ventilation. Peripheral ischemia was observed in a glove-and-stocking distribution in Patient 6. It fluctuated over time, independently of the changing vasopressor dose. Most of the peripheral ischemia slowly resolved, except in patch-

es of necrosis on the fingers of both hands and all the toes.

Over the next 30 days, all patients had generalized desquamation (most marked in Patients 5 and 6) and muscle weakness on discharge from the ICU. Five patients had late myalgia, headache after the discontinuation of corticosteroids, difficulties with concentration, and short-term difficulties in finding words (particularly names). Three patients had delayed hyperalgesia, and two had peripheral numbness. None had documented lymphadenopathy or splenomegaly while in the ICU or after discharge.

HEMATOLOGIC AND IMMUNOLOGIC PROGRESSION

The laboratory values for the six patients are summarized in Figure 3; data on the clinical course of each patient are provided in the Supplementary Appendix. Severe thrombocytopenia was observed, initially accompanying disseminated intravascular coagulation but persisting even after the other clotting values normalized (Fig. 3A, and Fig. 4A and 4B through 9A and 9B in the Supplementary Appendix). All patients had mild normocytic anemia that persisted beyond discharge from the ICU, followed by a slow recovery. Neutrophil numbers initially were preserved and then increased in response to corticosteroids (Fig. 3B), but they were dysplastic in appearance (Fig. 2C and 2D), a feature that eventually resolved. By contrast, marked lymphopenia and monocytopenia were noted in all patients 8 hours after TGN1412 infusion (Table 2).

Lymphocyte numbers were too low to allow for the measurement of cell subgroups 1 day after infusion. Subsequent blood tests showed increasing levels of CD4+ and CD8+ T cells (Fig. 3 through 9 in the Supplementary Appendix), CD19+ B cells, and CD16+ presumed natural killer cells, starting 48 hours after infusion. In Patients 1, 2, 3, and 4, who recovered the most rapidly, T-cell recovery occurred in a CD4+:CD8+ ratio of 1:1, with a temporary rise to levels just above normal in two patients (Fig. 4D and 7D in the Supplementary Appendix). Patients 5 and 6, the two who were most severely ill, had a slower recovery, with lower overall numbers of T cells (Fig. 8 and 9 in the Supplementary Appendix) and a CD4+:CD8+ ratio of 2:1.

The lymphocyte and monocyte nadirs in each patient occurred within 24 hours after TGN1412 infusion, overlapping with the cytokine storm (Fig. 3B, and Fig. 4C through 9C in the Supplementary Appendix). A dramatic increase in the level of tumor necrosis factor α (TNF-α) was observed in all patients within an hour after TGN1412 infusion, followed by elevations in the level of interleukin-2, 6, and 10 and interferon-γ within the first 4 hours after infusion (Fig. 3C, and Fig. 4C through 9C and 10 in the Supplementary Appendix). This cytokine release resolved after the first doses of hydrocortisone and methylprednisolone, and in Patients 1, 2, 3, and 4 the values normalized within 2 days. By contrast, in Patients 5 and 6, the cytokine storm was prolonged by 1 to 2 days; discrete elevations in the interleukin-6 and interleukin-4 levels, out of proportion to those noted in the other patients, were observed.

DISCUSSION

The intravenous infusion of TGN1412 in healthy persons produced a sudden and rapid release of proinflammatory cytokines. These unexpected clinical data provide insight into the natural course of the cytokine storm and the systemic inflammatory response syndrome (SIRS) in the absence of contaminating organic factors. Regulatory authorities, who tested TGN1412 from the same batch as the infused drug, found no errors in its manufacture, formulation, or administration and found no contamination with endotoxin, pyrogen, or microbiologic or other agents.[6] This type of cytokine release had not been observed in the preclinical studies of TGN1412, and it is currently unclear whether the severe effects of this type of cytokine release in vivo in humans is caused by the direct ligation of CD28 on T cells or by the ligation and activation of other cell types, leading to the release of preformed TNF-α, which then triggers the remainder of the cascade. The Secretary of State for Health has convened an expert scientific group to study the events of the clinical trial in greater detail.[6]

Clinically, the most striking phenomenon in the cohort was the stereotypical response to the study drug in all six patients and in all organ systems affected (albeit to varying degrees) (Table 3). All six patients initially had clinical signs that fit the criteria for SIRS.[8] Subsequently, the most prominent clinical feature was the early appearance of respiratory distress and pulmonary infiltrates, accompanied by renal impairment and

profound disseminated intravascular coagulation. This pattern of organ impairment may be consistent with a generalized multiorgan response to inflammation or critical illness.[9,10] However, the rapid onset and concordance of the lung injury among patients seemed unusual, and in the presence of high cytokine (especially interferon-γ and TNF-α) levels, these features may be consistent with immune-mediated injury that is specific to the lung.[11,12]

Alveolar macrophages in humans are normally inefficient in the costimulation of T cells through the CD28 pathway[13]; thus, our data suggest that anti-CD28 agonists in vivo may be able to potentiate immune activation and therefore lung injury. Neither cytokine storm nor lung injury was observed in the preclinical studies of TGN1412. This probably indicates that the presence of high levels of proinflammatory cytokines is a requirement for the pulmonary compromise, regardless of whether CD28 is ligated in the lung. In contrast to the pulmonary compromise that eventually ensues in SIRS, the more rapid onset of lung injury in our patients may have been due to the combination of the direct effects of the antibody and cytokines on lung tissue.

Equally striking was the consistent pattern of immunologic effects and recovery in all six patients. In particular, the severe lymphopenia observed in these patients was unexpected; a temporary lymphocytosis had been observed in preclinical studies of TGN1412 in animals.[3,4,14] This unanticipated lymphopenia in humans may have reflected cell death or the migration of cells to other tissues such as lymph nodes, although lymphadenopathy was not detected. Lymphopenia has been observed as part of the cytokine storm induced by other monoclonal antibodies.[15-17] However, the low cell numbers observed in these studies were anticipated, given the mechanism of action and the antilymphocyte specificity of the infused antibodies. Sepsis in humans may also induce lymphopenia that is selective for B cells and CD4+ T cells over the course of several days.[18] In contrast, the onset of lymphopenia within 8 hours after infusion of TGN1412, and the involvement of all mononuclear cells (CD4+ and CD8+ T cells and monocytes), may suggest that the depletion of cells in our patients was a response to the infused T-cell agonist drug rather than to the cytokine storm alone.

The clinical progression after infusion of TGN1412 can be separated into four phases (Fig. 1). Phase 1 began within an hour after infusion, continued through days 1 and 2 (and day 3, in Patients 5 and 6), and consisted of the cytokine storm, involving the rapid induction of type 1 and type 2 cytokines (to varying degrees) and severe lymphopenia and monocytopenia. Phase 2, the reactive phase, occurred from day 1 through day 3 (or days 1 through 8 in Patients 5 and 6, who were the most seriously ill); it consisted of renal failure, disseminated intravascular coagulation, pulmonary infiltrates, and respiratory failure. Phases 1 and 2 overlapped; phase 2 was not necessarily directly caused by the events in phase 1. The recovery phase, phase 3, occurred between day 3 and day 15 (or between day 5 and day 20, for the patients who were the sickest) and was

Table 3. Common Features after Infusion of TGN1412.

System	Feature
Cardiovascular	Capillary leak Hemodynamic instability Lactic acidemia
Renal	Early acute renal impairment Urinary sediment 10–100 White cells <10 Red cells Granular casts (two patients)
Pulmonary	Acute pulmonary changes (six patients) Met criteria for acute lung injury (two patients)* Met criteria for acute respiratory distress syndrome (one patient)*
Hematologic and immunologic	Cytokine storm (TNF-α; interferon-γ; interleukin-10, 6, 2) Increased C-reactive protein level and erythrocyte sedimentation rate Lymphopenia Monocytopenia Thrombocytopenia Disseminated intravascular coagulation Normochromic, normocytic anemia Dysplastic neutrophils but preserved numbers
Hepatic	Increased alanine aminotransferase and alkaline phosphatase levels
Integumentary	Diffuse erythema Late desquamation
Neurologic	Delirium Partial amnesia Paresthesia or localized numbness Difficulty concentrating (late) Headaches (early and late)
Autonomic, gastrointestinal, or both	Bowel urgency or diarrhea Nausea or vomiting
Musculoskeletal	Myalgia in lower back (early) and calves (late)

* Criteria are from the American–European Consensus Conference on ARDS.[7]

characterized by the recovery of renal and pulmonary function. This recovery was reflected in thrombocytosis and increases in alanine aminotransferase and monocyte and lymphocyte levels (mostly in a 1:1 ratio of CD4+:CD8+ T cells). The last phase, phase 4, can be described as a plateau or steady-state phase. It began 15 days after infusion (or 20 days after in Patients 5 and 6) and consisted of normalization of the measured variables. As compared with reactions to the infusion of other immunomodulatory agents (such as anti-CD20,[15] anti-CD3,[16] and anti-CD52 monoclonal antibodies[17]), the response to TGN1412 initially had similar kinetics, including the rapid increase in the levels of first TNF-α and then interferon-γ and interleukin-6, followed by cardiovascular instability, and disseminated intravascular coagulation. However, from phase 2 onward, features unique to the response to TGN1412 were apparent — including early acute lung injury, diffuse erythema with late desquamation, neurologic sequelae, and post-illness myalgias (Table 3).

These events occurred during the first dosing interval in a phase 1 drug trial of a humanized immunomodulatory monoclonal antibody involving healthy subjects. The events provide insight into an immune-mediated cytokine storm leading to multiorgan failure in the absence of infection, contamination with endotoxin, or underlying disease. The TGN1412 variant of the syndrome had some features that set it apart from a typical cytokine storm, most notably early acute lung injury and marked lymphopenia.

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REFERENCES

1. Luhder F, Huang Y, Dennehy KM, et al. Topological requirements and signaling properties of T cell-activating, anti-CD28 antibody superagonists. J Exp Med 2003;197:955-66.
2. Rodriguez-Palmero M, Hara T, Thumbs A, Hunig T. Triggering of T cell proliferation through CD28 induces GATA-3 and promotes T helper type 2 differentiation in vitro and in vivo. Eur J Immunol 1999; 29:3914-24.
3. Beyersdorf N, Gaupp S, Balbach K, et al. Selective targeting of regulatory T cells with CD28 superagonists allows effective therapy of experimental autoimmune encephalomyelitis. J Exp Med 2005;202:445-55.
4. TGN1412 investigator's brochure. Wurzburg, Germany: TeGenero Immunotherapeutics, 2005. (Accessed August 11, 2006, at http://www.mhra.gov.uk/home/idcplg?IdcService=GET_FILE&dDocName=CON2023518&RevisionSelectionMethod=LatestReleased.)
5. Offner PJ, Moore EE. Lung injury severity scoring in the era of lung protective mechanical ventilation: the PaO2/FiO2 ratio. J Trauma 2003;55:285-9.
6. Investigations into adverse incidents during clinical trials of TGN1412. London: Medicines and Healthcare products Regulatory Agency (MHRA), 2006. (Accessed August 11, 2006, at http://www.mhra.gov.uk/home/idcplg?IdcService=GET_FILE&dDocName=CON2023821&RevisionSelectionMethod=LatestReleased.)
7. Bernard GR, Artigas A, Brigham KL, et al. The American-European Consensus Conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trial coordination. Am J Respir Crit Care Med 1994;149:818-24.
8. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992;20:864-74.
9. Hudson LD, Milberg JA, Anardi D, Maunder RJ. Clinical risks for development of the acute respiratory distress syndrome. Am J Respir Crit Care Med 1995; 151:293-301.
10. Brivet FG, Kleinknecht DJ, Loirat P, Landais PJ. Acute renal failure in intensive care units — causes, outcome, and prognostic factors of hospital mortality: a prospective, multicenter study. Crit Care Med 1996;24:192-8.
11. Theron M, Huang KJ, Chen YW, Liu CC, Lei HY. A probable role for IFN-gamma in the development of a lung immunopathology in SARS. Cytokine 2005;32:30-8.
12. Gon Y, Wood MR, Kiosses WB, et al. S1P3 receptor-induced reorganization of epithelial tight junctions compromises lung barrier integrity and is potentiated by TNF. Proc Natl Acad Sci U S A 2005; 102:9270-5.
13. Chelen CJ, Fang Y, Freeman GJ, et al. Human alveolar macrophages present antigen ineffectively due to defective expression of B7 costimulatory cell surface molecules. J Clin Invest 1995;95:1415-21.
14. Hunig T, Dennehy K. CD28 superagonists: mode of action and therapeutic potential. Immunol Lett 2005;100:21-8.
15. Winkler U, Jensen M, Manzke O, Schulz H, Diehl V, Engert A. Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (rituximab, IDEC-C2B8). Blood 1999;94:2217-24.
16. Gaston RS, Deierhoi MH, Patterson T, et al. OKT3 first-dose reaction: association with T cell subsets and cytokine release. Kidney Int 1991;39:141-8.
17. Wing MG, Moreau T, Greenwood J, et al. Mechanism of first-dose cytokine-release syndrome by CAMPATH 1-H: involvement of CD16 (FcgammaRIII) and CD11a/CD18 (LFA-1) on NK cells. J Clin Invest 1996;98: 2819-26.
18. Hotchkiss RS, Tinsley KW, Swanson PE, et al. Sepsis-induced apoptosis causes progressive profound depletion of B and CD4+ T lymphocytes in humans. J Immunol 2001;166:6952-63.