WHOLE bacteria or bacterial components or their extracts were employed to restore or augment the immune system. Beneficial effects were attained with these agents in treating various diseases. These agents were named biological response modifiers (BRMs) because they regulated certain cellular components of the immune system. The cellular regulation induced by these BRMs was found to be due to cytokines. The cytokines were shown to act directly on the various cellular components and to provide therapeutic benefit in various autoimmune and immune deficiency diseases. Overproduction of specific cytokines however leads to a deleterious effect on the host. Overproduction of tumour necrosis factor (endotoxin, lipopolysaccharide) leads to septic shock. Bacteraemia is the leading cause of overproduction of tumour necrosis factor (TNF). Septic shock in many cases leads to death. Several monoclonal antibodies to lipopolysaccharide (LPS) and anticytokines have demonstrated protection against septic shock.

Key words: Biological response modifiers, Cytokines, Interleukins, Septic shock, Tumour necrosis factor

Immunoregulatory biological response modifiers: effect of cytokines on septic shock

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A little over 25 years ago the concept of introducing an exogenous substance to elicit a host immune response was limited to such agents as bacterial organisms (Table 1), to be followed a few years later by the use of partially purified microbial extracts or fractions (Table 2). The use of these microbial agents to stimulate the immune response gave way to the use of chemically defined BRMs (Table 3), and to extracts from yeast, fungi, plants and herbs (Table 4), and polysaccharides (Table 5).^{1,2}

During the past 15 years, as the result of progress made in molecular biology, the immunoregulating effects attributed to the aforementioned BRMs were found to be due to cytokines that were induced and secreted by cells responding to these BRMs (Table 6).

The capacity of several of these BRMs to regulate a specific cell population and/or induce the production of a specific cytokine and its therapeutic

use for various diseases is shown in Tables 7 to 11. Table 7 shows that the use of BCG increased the various cellular responses: T-cells, macrophages (MPG), natural killer cells (NK), and cytotoxic T-lymphocytes (CTL). The cytokines induced by BCG are interleukin 1 (IL-1), IL-2, interferon alpha (IFN- α), granulocyte-monocyte colony stimulating factor (GM-CSF), and tumour necrosis factor (TNF). Treatment with BCG alone, or combined with other treatment modalities (chemotherapy, surgery, irradiation), resulted in beneficial responses in several animal and human cancers. BCG

Table 1. Biological response modifiers of biological origin

Microorganisms (bacterial preparations)	
<i>Bacillus Calmette–Guerin</i> (BCG)	<i>Haemophilus influenza</i>
<i>Corynebacterium parvum</i>	<i>Streptococcus pyogenes</i>
<i>Brucella abortus</i>	<i>S. aureus</i> , <i>S. viridans</i>
<i>Bordetella pertussis</i>	<i>Neisseria catarrhalis</i>
<i>Nocardia rubra</i>	<i>Salmonella typhimurium</i>
<i>Pseudomonas aeruginosa</i>	<i>Listeria monocytogenes</i>
<i>Chlorella vulgaris</i>	<i>Streptomyces folvoviridis</i>
<i>Klebsiella pneumonia</i>	<i>Serratia marcescens</i>
<i>Diplococcus pneumonia</i>	<i>Chlorella vulgaris</i>

Table 2. Biological response modifiers of biological origin

Microorganisms (complete or partially pure extracts)
<i>Picibanil</i> (OK432, <i>Streptococcus pyogenes</i>)
MER (methanol extracted residue, BCG)
MY-1 (extract BCG)
RU41740 (Biostim, glycoprotein, <i>K. pneumoniae</i>)
Mycolic acid (glycolipid, <i>Nocardia rubra</i>)
Bru Pel (<i>Brucella abortus</i>)
MDP (muramyl dipeptide, <i>Mycobacterium smegmatis</i>)
WSA (disaccharide peptoglycan, <i>Mycobacterium smegmatis</i>)
CWS (cell wall skeleton, BCG, <i>Nocardia rubra</i>)
Forphenicino 1 (<i>Aspergillus fulvoviridis</i>)
GBA (amino sugar polymer, cytophaga)
BV (Broncho Vaxom, alkaline lysis extract of eight bacterial mixtures)
Imuvert (ribosomes and membrane vesicles of <i>Serratia marcescens</i>)
Detox (detoxified endotoxin)
OM-89 (purified <i>E. coli</i> extract)
CVE (glycoprotein, H ₂ O extract of <i>Chlorella vulgaris</i>)
SSG (β 1,3 glucan, <i>Sclerotinia sclerotiorum</i>)
Other fungal extracted β 1,3 glucans (sonifilan, sizofiran, grifolan)

Table 3. Chemically defined biological response modifiers

Amphotericin B	HAB 439	Splenopentin (DACP-5)
Azimexon	Imuthiol	SPG
ADA 202-718	Indomethacin	THF ₂
ABPP	Isoprinosine	TOK 8801
A 171-172	LF 1695	Thymopentin
Bestatin	L 646,257	Tuftsia
Betafectin	Levamisole	Therafectin (SM 1213)
Cimetidine	Lipidal amines	Thiazolobenzimidazole
CL 246,738	MDPs	Thiobendazole
CL 259,763	MPG	IFN inducers:
DT 5461	MIMP	Poly IC
EL-1, EL-2	Pyrimidinols	Poly ICLC
Formosanin-C	Retinoids	Poly AV
Forphenicidinol	Ricin	Ampligen
FK 506, FK 565,	SM 8849	Anthroquinones
FK 156	SDZ MRL 953	RP 40639 Pimelantide
RU 41740 (Biostim)	Oxamisole	

treatment of human transitional cell carcinoma of the bladder led to greater than 90% regression with minimal recurrence. More recently local/regional immunotherapy with IL-2 was reported to also mediate regression of this tumour type.³ This response demonstrates that the cytokine inducer (BCG), as well as the BCG induced cytokine (IL-2) were both therapeutically effective against the same tumour.

OK 432 (Table 7) has had extensive preclinical and clinical evaluation⁴⁻⁶ and has been shown to be beneficial in treating various cancers. Similarly, Lentinan has shown efficacy in augmenting immune restorative activity beneficial for treating various infections.⁴ Tables 8 to 11 describe several BRMs of bacterial, thymus, and polysaccharide origin which exert immunoregulatory activity.

Swainsonine (Table 11) was recently reported to cause a marked elevation in superoxide radical

Table 4. Extracts from yeast, fungi, plants and herbs**Extracts isolated from yeast or fungi**

Krestin (PSK, protein bound polysaccharide, *Coriolas versicolor*)
 Glucan (beta 1,3 polyglucose, *Saccharomyces cerevisiae*)
 Lentinan (beta 1,3 polyglucose, *Lentinus edodes*)
 Cyclomunine (cyclohexa peptide, *Fusarium equiseti*)
 Bestatin (2S, 3R-amino-2-hydroxy-4-phenylbutanoyl-L-leucine, *Streptomyces olivoreticuli*)
 Schizophyllan (beta 1,3 polyglucose, *Schizophyllum commune*)
 SPG (sulphated schizophyllan)
 FK156 and FK565 (tetrapeptides, *Streptomyces olivaceo-griseus*)
 AM-3 (protein complexed beta 1,6 polyglucan, *Candida utilis*)

Plant and herb extracts

Ace mannan (Carrisyn, *Aloe vera barbadensis*)
 Bryostatin (macrocyclic lactones, marine plant *Bryozoa bugula meritina*)
 RBS (RON) (α -glucan derived from rice bran)
 Formosanin-C (diosgenin saponin, *Paris formosana hayata* plant, *Liliaceae*)
 Ginseng (*Panax ginseng*, *Araliaceae*)
 Huang-chi (*Astragalus membranaceus*, *Leguminosae*)
 Shosaiko-To (Xiao-Chai-Hu-Tang, seven herb mixture)

Table 5. Polysaccharide biological response modifiers

AM3
β -1,6, polyglycan protein (<i>Candida albicans</i>)
Betafectin
poly (1,6) β -D-glycopyranosyl-(1,3) β -D-glucopyranose (<i>Saccharomyces cerevisiae</i>)
Curdlan sulphate (CS)
sulphated β -1, 3-D-glucopyranan
Glucan
β -1, 3 glucose (<i>Saccharomyces cerevisiae</i>)
GBA
N-acetyl glucosamine (<i>Cytophaga bacterium</i>)
Lentinan
β -1,3-glucan, 1-6- β -D glucopyranoside (<i>Lentinus edodes</i> , mushroom)
PSK (Krestin)
protein bound polysaccharide (<i>Coriolus versicolor</i> , mushroom)
RBS (RON)
α glucan derived from rice bran
RU41740 (Biostim)-MPG
glycoprotein extract (<i>Klebsiella pneumoniae</i>)
SDZ MEL 953
synthetic acylated glucosamine-monosaccharide-1-PO ₄
SSG
β -1,3 D glucan (<i>Sclerotinia sclerotiorum</i> , fungus)
Sizofiran
β -polyglucan (<i>Schizophyllum commune</i>)
SPG
β -1,3 glucan (Sulphated <i>Schizophyllum</i>)
Sonifilan
β -1,3 glucan (fungal extract)
SPR 901
α -1,6 glucan (<i>Leuconostoc mesenteroides</i>)
WSA
disaccharide bound to peptidoglycan (<i>M. smegmatis</i>)

Table 6. Interrelationship of biological response modifiers to cytokines

Table 7. Characterization of biological response modifiers relative to cell regulated, cytokine and disease treatment**Bacterial****BCG**

CR: ↑ T-cell function, macrophage (MPG), NK, CTL
 CK: IL-1, IL-2, IFN- α , GM-CSF, TNF α

TR: + murine antitumour effect $\pm$ adjuvant therapy; human recurrent superficial bladder tumours, transurethral resection and intravesical BCG leads to greater than 90% response, absence of recurrence.

OK432 (Pecibinil) *Streptococcus pyogenes*, strain A3

CR: ↑ T-cell function, MPG, NK, CTL, TIL

CK: IL-1, IL-2, IFN γ , TNF

TR: + murine antitumour; increase survival time and disease free interval in human colo-rectal, cervical, lung Ca; ↑ NK, MPG, T-cells in malignant ascites.

Lentinan (1,3, β -D-glucan; 1,6- β -D-9-glucopyranosides), *Lentinus edodes*

CR: NK, MPG, T, bone marrow

CK: IL-2, IFN, CST

TR: + vs murine tumours, increase in ST, duration of remission of human CA, ↑ efficacy with chemical prophylactic vs influenza; *Mesocostoides corti* prophylaxis.

CR = cell response; CK = cytokines; TR = therapeutic response.

production and induction of TNF- α secretion from macrophages.⁷ Of particular interest is swainsonine's protective effect against bone marrow depression resulting from treatment with the chemotherapeutic agents methotrexate (MTX), 5-fluorouracil (5-FU), cyclophosphamide (CP), or doxorubicin (DOX).

Thymosin fraction 5 (THF 5) when combined with thymosin alpha for the treatment of chronic hepatitis B led to clearance of the virus and improved liver function.

Table 8. Biological response modifiers of bacterial origin**Bacterial extracts****RU41740 (Biostim, MPG) membrane protein extract of *Klebsiella pneumonia***

CR: T, MPG, NK, polyclonal B

CK: IL-1, IL-6, GM-CSF

TR: prevents bacterial respiratory infection

FK 156, 506, 565: original 156 isolated from fermentation broth of *Streptomyces* FK 156, 565

CR: T, NK, MPG

CK: IL-1, CSF

TR: + murine tumours, antiviral (antibacterial)

Treatment	Dose μ g/kg	NK Cytot. 100:1	No. lung mets; B16
Saline	—	10.1	287
FK565	0.01	9.9	307
	0.1	34.3	62
	1.0	29.0	9

FK506: immunosuppressor: mechanism is considered to operate during the first signal transduction cascades and ultimately blocks the transcription of the genes that encode IL-2 and other lymphokines. Reported effective in treating psoriasis, organ rejection, nephrotic syndrome, Type 1 diabetes (insulin-dependent juvenile diabetes).

CR=cell response; CK = cytokines; TR = therapeutic response.

Table 9. Biological response modifiers of thymus and various origin**Thymomodulin (TMD), thymolymphotropin (TLT), thymic derivatives containing several peptides**

CR: NK, T, B, MPG

CK: IFN- γ , IL-2, BCGF, GM-CSF

TR: ↑ CD4 in AIDS; 120 mg/kg/day per o.s. for 30 d in acute type B hepatitis led to accelerated AST, ALT decrease, early HBs Ag clearance and normalization of elevated cDg; a 50% sero conversion of pediatric HBs Ag⁺ H-Be Ag⁺ chronic liver disease; and, protection vs bone marrow depression during intensive antitumour chemotherapy.

Betafectin—poly 1,6 β -D-glucopyranosyl/1,3, β -D glucopyranose (glucose polymer); *Saccharomyces cerevisiae* RAD2.

CR: ↑ MPG, NK, BM

CK: IL-1, IFN

TR: Protects vs *E. coli* peritoneal sepsis, *Candida albicans*, *S. Aureus*, lethal irradiation LD₉₀. Mechanism binds to β -glucan receptors on Mo, PMN, which enhance phagocytic and microbicidal activity.

AM-3—protein complexed B1,6, polyglucan, *Candida utilis*

CR: NK, T, MPG, bone marrow

CK: IL-1, IL-2, IFN $\alpha\beta$, CSF

TR: + vs murine tumour and viral infection; combined with chemotherapy led to earlier reconstitution of bone marrow, decrease in secondary infections.

CR = cell response; CK = cytokines; TR = therapeutic response.

Another example of the similarity in responses achieved by BRMs and the cytokines they induce is shown in Table 12. Ampligen and CL246,738 are potent interferon inducers. Their antiviral effects were compared to recombinant IFN gamma (rIFN- γ) and rIFN- α against four virus types. All four agents augmented NK cell activity as well as activating macrophages. An antiviral correlation was shown among all four against three of the four viruses.

Cytokines (Table 13) are increasingly recognized as essential and powerful cell communication and

Table 10. Biological response modifiers of various origin**Synthetics****Oxamisole—2,3,5,6,7,8 hexahydro - 2 - phenyl - 8,8 - dimethoxy - imidazole [1,2, α] pyridine**

CR: NK, ADCC

CK: IL-1, IL-2

TR: effective vs murine hepatitis (FB strain) decreased hepatic discoloration, SGOT, SGPT, virus titre in liver

Ampligen—mds RNA

CR: NK, MPG, CD4

CK: IFN $\alpha\beta$, CSF

TR: + vs murine tumours; inhibits AZT sensitive and resistant HIV-1, protects vs AZT bone marrow toxicity, increased CD4 count in treated AIDS patients.

HAB439—3 phenyl-2-isoxazoline-5-yl phosphoric acid

CR: T, NK

CK: not known

TR: may act through an aminopeptidase inhibitor; inhibits *Listeria monocytogenes*, *Salmonella typhimurium*, synergistic with ampicillin.

CR = cell response; CK = cytokines; TR = therapeutic response.

Table 11. Current applications of biological response modifiers

Swainsonine—indolizidine alkaloid CR: NK, MPG, bone marrow CK: IL-1, IL-2 TR: protects vs drug induced bone marrow suppression, LD ₉₀ doses MTX, 5FU, CP, DOX combined with drug led to stimulation of bone marrow.
Thymosin—thymosin fraction 5 + thymosin alpha 1. TR: treatment of chronic hepatitis B (phase II). Patients (20) enrolled; histological and biochemical evidence for active liver disease with + serum hepatitis B virus (HBV) DNA + hepatitis B surface antigen (HBsAg). Rx, 2x/week, 6 months sc. At 1 year: 9/12 thymosin treated and 2/8 placebo showed normal serum alanine aminotransferase (ALT) and cleared (HBV) DNA from serum ($p < 0.04$). From twelve available pre- and post-treated liver biopsies; 4/5 placebo and 1/7 thymosin Rx livers possessed replicative forms of (HBV) DNA.

CR = cell response; CK = cytokines; TR = therapeutic response.

Table 12. Antiviral—*in vivo* responses

BRM	NK	MPG	VEE	BANZI	HSV2	Caraparu
rIFN- γ	+	+	+	—	+	NT
rIFN- α	+	+	+	+	+	NT
Ampligen	+	+	—	+	+	—
CL246,738	+	+	+	+	+	+

VEE = *Venezuelan equine encephalitis* (alpha toga virus); BANZI (flavivirus); Caraparu (bunyavirus). + positive response; — no response; NT not tested.

regulatory molecules. They are implicated in normal homeostasis and defence against many diseases and are involved in the pathogenesis of a variety of disorders. As a result of their powerful and widespread regulatory functions, a number of cytokines are being tested as therapeutic agents (Table 14). At the same time, many antagonistic factors are being developed for use in those diseases that may be initiated or mediated by cytokines.

Table 14. Cytokines and their applications

EPO (Erythropoietin). Regulates RBC production. Used for treating anaemia (reduces or eliminates blood transfusions in kidney dialysis patients, AIDS, cancer).
G-CSF (Granulocyte Colony Stimulating Factor). Stimulates granulocyte formation, adjuvant use to overcome leukopenia developing after chemotherapy.
GM-CSF (Granulocyte-Macrophage Colony Stimulating Factor). Stimulates production and function of activities of granulocytes and macrophages. Uses are similar to G-CSF.
IL-2 (Interleukin 2). Stimulates production and function of T-cells, NK cells. Use in treating renal cell carcinoma, melanoma. Potential synergy with alpha IFN; uses in infection and vaccine adjuvant.
Alpha Interferon. Approved in 50 countries for 16 indications including cancer, AIDS, hepatitis.
Beta Interferon. Similar clinical properties to alpha-IFN. Being tested for viral diseases and multiple sclerosis.
Gamma Interferon. Potential uses in cancer, infectious diseases, ectopic dermatitis, rheumatoid arthritis.
TNF (tumour necrosis factor). rTNF used in limited clinical trials as potential anti-cancer agent.

The field of cytokine biology is growing rapidly. Studies are being conducted that embrace analysis of molecular regulation of cytokine expression through the use of cytokines *per se* as therapeutic agents. Diseases that involve employing cytokines include cancer, infectious diseases, inflammatory processes, autoimmune disorders, physiological development defects, and ageing. Cytokines appear to extend in some fashion to every organ system of the body. Of particular interest is the potential use of cytokines in the treatment of cancer, where they are being tested as direct antitumour agents, as enhancers of various cellular components of the immune system reactive against tumour cells, as potentiators of chemotherapy and radiotherapy, and as protective agents against the myelotoxicity

Table 13. Cytokines derived from monocytes/macrophages (monokines) or lymphocytes (lymphokines)

Cytokine	Primary cell source	Primary effects
IL-1	Macrophages, keratinocytes, endothelial cells, fibroblasts, T, B	Inflammatory, haematopoietic
IL-2	T-lymphocytes and LGL	Activates T- and NK cells
IL-3	T-lymphocytes	Promotes myeloid progenitor cells
IL-4	Th cells	T-, B-cell growth factor, promotes IgE
IL-5	Th cells	Stimulates B-cells, eosinophils
IL-6	Fibroblasts, other	Growth factor B-cells, polyclonal IG
IL-7	Stromal cells	Lymphopoietin, generates pre-B, T
IL-8	Macrophages, other	Regulates lymphocyte, neutrophils, homing, infiltration, chemo attracts
G-CSF	Monocytes, other	Myeloid growth factor, generates neutrophils
M-CSF	Monocytes, other	Macrophage growth factor, macrophages
GM-CSF	T-cells, other	Monomyelocytic GF, myelopoiesis
IFN- α	Leucocytes	Stimulates macrophages and NK cells
IFN- β	Fibroblasts	Stimulates macrophages and NK cells
IFN- γ	T- and NK cells	Induces cell membrane Ag (MHC)
TNF α	Macrophages, other	Vascular thromboses, tumour necrosis
TNF β -LT	T-lymphocytes	and inflammatory, immunoenhancer
TNF β	Platelets, bone, other	Wound healing, bone remodelling, fibroplasia, immunosuppressor

Table 15. Mediators of natural immunity

Cytokine	Cell source	Cell target	Primary effects
Type 1 IFN	Mononuclear phagocyte (a), fibroblast (b)	All	Antiviral, antiproliferative; increases class I MHC expression
Tumour necrosis factor 1	Mononuclear phagocyte, T-cell	Neutrophil, endothelial, hypothalamus, liver, muscle, fat, T-cell, B-cell	Activation (inflammation), activation (coagulation), fever, acute phase reactants, catabolism (cachexia), costimulator
Interleukin 1	Mononuclear phagocyte, other	T-cell, B-cell, endothelial cell, hypothalamus, liver, muscle, fat	Costimulator, antiviral (inflammation, coagulation), fever, acute phase reactants, catabolism (cachexia)
Interleukin 6	Mononuclear phagocytes, T-cell, endothelial cell	T-cell, B-cell, mature B-cell, liver	Costimulator, growth, acute phase reactants
MIP-1	Mononuclear phagocytes	Neutrophils, macrophages, hypothalamus	Costimulator, activation (inflammation), costimulator (induced TNF α) IL-1, IL-6 production), fever

associated with current cancer treatments. In contrast to their potential therapeutic value, some cancer cells produce cytokines which may be involved in autocrine growth stimulation.

Cytokines have been classified into four categories:

- (1) Mediators of natural immunity consisting of Type 1 IFN, TNF, IL-1, IL-6, and low molecular weight inflammatory cytokines to which the recently reported MIP-1 may belong.⁸
- (2) Mediators of lymphocyte activation, growth, and differentiation consisting of IL-2, IL-4 and transforming growth factor beta (TGF- β).
- (3) Mediators of effector cell activation consisting of IFN- γ , lymphotoxin, IL-5, and migration inhibition factor (MIF).
- (4) Mediators of immature leucocyte growth and differentiation consisting of IL-3, GM-CSF, M-CSF, G-CSF, and IL-7.

Mediators of natural immunity are the host's more initial and immediate responders since they initiate protection against viral infection. They initiate inflammatory reactions that protect against bacteria (Table 15). In some cases this complex defence network successfully restores normal

homeostasis and health, but at other times the overproduction of immunoregulatory mediators may actually prove deleterious to the host. Some examples of immune system mediated injury have been extensively investigated including anaphylactic shock, autoimmune disease, and immune complex disorders. More recently it has become clear that the cytokine TNF occupies a key role in the pathophysiology associated with diverse inflammatory states and other serious illnesses including septic shock. TNF exhibits beneficial and deleterious functions when combined with adriamycin.⁹ TNF demonstrated an enhanced cytotoxicity against several adriamycin resistant human tumour lines. A similar synergistic enhancement of adriamycin cytotoxicity against human osteosarcoma cells by TNF- α was also reported.¹⁰

In contrast, several reports indicate that TNF also possesses deleterious properties. Recombinant TNF functioned as an autocrine growth factor which enhanced growth of four human neuroblastoma cell lines.¹¹ Blocking factors (soluble receptors) for human TNF and lymphotoxin (LT) were found in the serum, cerebral spinal fluid, and tumour cyst fluid (TCF) of human glioma tumour patients.¹² The presence of these receptors would abrogate any tumour cytolytic activity of TNF or

Table 17. Potential treatments for septic shock

Early attempts

1980s Treatment with human antisera to *E. coli* J5, a mutant strain producing LPS with variable oligosaccharide. Of 212 bacteraemic patients treated 50% survived.

Drawbacks to antiserum—heterologous pool of sera, standardization difficulty, serum contaminated with infectious agents.

1985 Monoclonal immunoglobulin M (IgM) binding specifically to the lipid A domain of LPS. Comparative study:

1. Standard therapy and antibiotics and fluids, 49% died.
2. Standard therapy and mAb to lipid A, 30% died.

1990s

1. IL-1ra (IL-1 receptor antagonist). Natural protein antagonist of IL-1, competes for IL-1 receptor (Antril).
2. IL-1r (interleukin 1 receptor). Competes with the natural IL-1 receptor to down-regulate IL-1.
3. Anti-TNF mAb (antitumour necrosis factor antibody). Blocks TNF mediated damage in sepsis, cachexia, etc.
4. TNF inhibitors (tumour necrosis factor inhibitors). Natural protein inhibitors of TNF that might limit systemic damage in septic shock.
5. TNFr-Fc (TNF receptor immunoadhesion). Soluble TNFr linked to Fc region of mAb. Potential use in sepsis.
6. Sepsis mAb. Monoclonal antibodies vs Gram-negative bacterial endotoxin for treatment of sepsis syndrome and septic shock.
7. BPI (bacterial permeability increasing protein). Neutrophil granule protein, blocks Gram-negative endotoxin; inhibits TNF, IL-6 and IL-8 cytokines.
8. Other drugs: pentoxifylline; L-carnitine derivatives.

LT. Similar TNF growth promoting activities were reported for ovarian cancer¹³ and perhaps for mammary tumour cells.¹⁴ Colony growth of leukaemic colony-forming units (L-CFU) obtained from patients with primary acute myelogenous leukaemia stimulated with recombinant human IL-3 (rhIL-3) was reported to be significantly potentiated when recombinant human TNF- α was present in the culture.¹⁵ Studies on the mechanism of action of rhTNF- α on the acute myelogenous leukaemia L-CFU growth suggests that TNF- α acts by inducing release of growth stimulating haematopoietic cytokines by the leukaemic cells themselves including IL-1 α , IL-1 β , GM-CSF, G-CSF, and IL-6. Treatment with neutralizing antibody to all of the above cytokines significantly inhibited the synergistic interplay of TNF- α and IL-3. The role of TNF leading to septic shock is illustrated in Table 16.

Advances in cytokinology and molecular biology develop in such a way that multiple approaches emerge to attack a problem. That has happened for septic shock, a frequently fatal reaction following bacterial infection leading to a bacteraemia. Using recent advances in cytokine biology and recombinant DNA technology immunologists and molecular biologists are currently evaluating six potential new treatments for septic shock.¹⁶

Investigators who study septic shock fall into two categories (Table 17). One group works to

Table 16. Tumour necrosis factor leading to septic shock

The most prominent bacterial infections are *E. coli*, *P. aeruginosa*, *Klebsiella* and *Bacteroides*. When these are present in blood (bacterial sepsis), levels of TNF increase, and autocrine and paracrine effects increase IL-1 and IL-6. The combined effect

1. Leads to depressed myocardial contractility (reduces tissue perfusion).
2. Relaxes vascular smooth muscle tone (leads to a reduction of blood pressure and tissue perfusion).
3. Causes intravascular thrombosis.
4. Causes severe metabolic disturbances (life threatening fall in blood glucose concentrations).
5. Leads to a spontaneous clotting in blood vessels, severe hypotension, multiple organ damage (mostly due to circulatory collapse and oxygen free-radical damage).

The rate of occurrence of septic shock following septicaemia is 175 per 100 000 people yearly, and 500 per 100 000 for patients in hospitals. Of those that develop septic shock, 25 to 40% die.

interrupt the cytokine mediated immune response to septicaemia, while the other group targets LPS, either to neutralize it in the blood or to prevent it from activating leucocytes that start the inflammatory response. The latter approach however would function for Gram-negative sepsis only.

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