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IMMUNOMODULATION WITH LIPOSOMAL MTPPE AND IFN-γ IN GRAM-NEGATIVE SEPTICAEMIA

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INTRODUCTION

Severe infections represent a continuing threat to patients. The disappointing results with antibiotics are most prominent in the immunodeficient host. Factors considered important in the development of septicaemia include broad-spectrum antibiotic therapy, immunosuppressive treatments, invasive devices and surgery, penetrating wounds, burns or other trauma, anatomic obstruction, intestinal ulceration, the increased average age, and the very young, as well as progressive clinical conditions (malignancies, diabetes, AIDS, and other serious chronic diseases). Acquired and congenital immunodeficiencies as well as disease-associated host defence disorders add to the problem of increased fatal bacterial infections.

The major factor contributing to the failure of antibiotics to adequately combat bacterial infections in the immunodeficient host is probably the lack of support by the host defence system especially those who had persistent neutropenia. Different methods are available to improve treatment. One method is the intensification of antibiotic treatment, for instance the application of more drugs at the same. Another possible way to improve the therapeutic results might be the stimulation of the non-specific host defence.

Activation of the non-specific host defence has some advantages. An important advantage is that immunomodulation can be effective in different types of infection, and compared with antibiotic treatment there is no induction of tolerance of the microorganisms to the treatment. Especially the cells of the mononuclear phagocyte system (MPS) play a key function in the non-specific host defence. Activation of these cells will result first of all in enhanced killing of intracellular microorganisms infecting the MPS. However, it is expected that activation of the MPS can also enhance the resistance to more systemic (extracellular) infections. Activation of the non-specific host defence can be achieved with immunomodulators: biological or synthetic agents that influence or modify (parts of) the innate resistance in a direct or indirect way, independent of the challenge. Many different agents are tested for their immunomodulatory capacity. The immunomodulatory agents are from natural origin, for instance extracts of bacterial or herbal origin and cytokines, or synthetic (for instance some of the muramyl peptide derivatives). Here we focus in particular on muramyl tripeptide phosphatidylethanolamine (MTPPE),

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and the cytokine interferon-γ (IFN-γ). MTPPE is a derivative of muramyl dipeptide (MDP), the smallest fragment of the petidoglycan with adjuvant activity. Macrophage stimulating and antimicrobial activity of MDP (and derivatives) and IFN-γ has already been shown *in vitro* and *in vivo* to a broad spectrum of microorganisms. Thus far promising studies with IFN-γ in chronic granulomatous disease (CGD) patients has led to the approval of IFN-γ for prophylaxis against opportunistic infections in these patients (*The International Chronic Granulomatous Diseases Cooperative Study Group*, 1991).

MURAMYL PEPTIDES AND INTERFERON-γ

MDP can be produced synthetically, and many different modifications are made to reduce toxicity or improve activity and usability. The derivative discussed here is MTPPE, a synthetic hydrophobic derivative of MDP, in the liposome-encapsulated form (LE-MTPPE). MTPPE shows improved activity over MDP and due to its hydrophobicity a greatly increased association with liposomes. IFN-γ is a cytokine primarily produced by TH1 cells, CD8+ T cells and NK cells upon stimulation by for instance IL-1 and IL-12, and has stimulating activity on macrophages. IFN-γ influences all major macrophage functions including MHC II expression, antigen presentation, FcR1 receptor expression, uptake and intracellular killing of microorganisms, tumour cell cytotoxicity, and the production of monokines (*Baron* et al., 1991; *Biliau* and *Dijkmans*, 1990; *Czarniecki* and *Sonnenfeld*, 1993; *IJzermans* and *Marguet*, 1989; *Murray*, 1988, 1992; *Williams* et al., 1993).

Both muramyl peptides and IFN-γ were found to have potent antimicrobial enhancing effect on macrophages. The agents were found to induce the production of reactive oxygen and nitrogen intermediates (ROI and RNI), and other antimicrobial agents by macrophages, explaining the enhanced antimicrobial activity of the infected cells. Therefore, the rational to use these immunomodulators for the activation of the host defence to intracellular infections *in vivo* is easy to understand. However, macrophage activity can be enhanced by the immunomodulators also with respect to extracellular infections. *In vitro* results are some times in contradiction, however, increased uptake and killing of *Pseudomonas aeruginosa* by macrophages exposed to IFN-γ *in vitro* was noticed (*Pierangeli* and *Sonnenfeld*, 1993). Treatment of human peripheral blood monocytes with IFN-γ *in vitro* greatly enhanced both respiratory burst and microbicidal activity towards *P. aeruginosa* (*Kemmerich* et al., 1987). Others demonstrated that IFN-γ had no effect on macrophage phagocytic capacity of *Staphylococcus aureus* (*Quiroga* et al., 1992), or was even shown to have a negative effect on the uptake and killing of *P. aeruginosa* and *S. aureus* by macrophages (*Speert* and *Thorson*, 1991). Culture of human monocytes in the presence of IFN-γ enhanced the capacity to produce superoxide anion. However, the phagocytosis of *P. aeruginosa* was substantially depressed in a dose dependent fashion (*Speert* and *Thorson*, 1991). These findings are supported by *in vivo* incubation of resident or exudate peritoneal macrophages with i.p. injected IFN-γ. IFN-γ did not result in increased *in vitro* phagocytosis of *Salmonella typhimurium* as compared with untreated mice (*van Dissel* et al., 1987). Similar results were obtained after 18 h of *in*

vitro incubation of resident or exudate peritoneal macrophages with IFN-γ.

Others found that peritoneal macrophages incubated with IFN-γ for 12 h exhibited enhanced bactericidal activity against *S. typhimurium* (*Kagaya* et al., 1989) or *Salmonella enteritidis* (*Sasahara* et al., 1992), independent of oxygen metabolism. These results suggest that increased generation of ROI may not be primarily responsible for the observed ability to inhibit intracellular growth of bacteria. It was observed in our laboratory that peritoneal macrophages exposed LE-IFN-γ or LE-MTPPE or these agents combined (LE-MTPPE/lFN-γ) resulted in increased production of both ROI and RNI. However, the peritoneal macrophages did not exhibit increased phagocytic activity towards *K. pneumoniae* (*ten Hagen* et al., 1995). In contradiction it was demonstrated that MDP-lys(L18), an MDP analogue, stimulated alveolar macrophages to phagocytise *P. aeruginosa* (*Ozaki* et al., 1989). Also peritoneal macrophages isolated form mice treated with MDP showed marked augmentation of the phagocytosis of *Escherichia coli in vitro* (*Friedman* and *Warren*, 1984). These results clearly show that exposure of macrophages *in vitro* to IFN-γ or MDP derivatives results in a heterogeneous response with respect to bacterial killing. The discrepancy found is probably not only due to the different bacteria used but also depends on macrophage culture purity and condition.

Administration of MDP or MTPPE, as well as IFN-γ was shown to stimulate the host resistance in mice to *K. pneumoniae* infection in several models. The host resistance could be enhanced by prophylactic intravenous or subcutaneous administration of muramyl peptides to intravenous and intramuscular infections with *K. pneumoniae* (*Ausobsky* et al., 1984; *Chedid* et al., 1977; *Melissen* et al., 1992; *Parant* et al., 1978). Also intragastric administration of MDP in mice infected with *K. pneumoniae* resulted in enhanced resistance when administered 7 days before challenge (*Parant* and *Chedid*, 1985) or intramuscular infection with *K. pneumoniae* (*Chedid* et al., 1977). However, oral administration is less potent when compared with intravenous administration. Orally administered MDP analogues were not active towards intraperitoneal *P. aeruginosa* infection, whereas these compounds intravenously, intraperitoneally or subcutaneously injected protected mice from the infection (*Fraser-Smith* et al., 1983).

Protective activity of MDP (the derivative MDP-lys(L18)) was also demonstrated against *E. coli* infections and in lesser amount against *P. aeruginosa* (*Matsumoto* et al., 1983; *Osada* et al., 1982). There was a significant reduction in mortality in mice intraperitoneally infected with *E. coli* and treated with MDP compared to the controls (*Cheadle* et al., 1989), which was shown by others to correlate with increased WBC count (*Stellato* et al., 1988). MDP analogues administered intraperitoneally one day before infection with *P. aeruginosa* enhanced nonspecific host resistance (*Furuya* et al., 1989). A significant reduction in *E. coli* bacteraemia was observed in animals treated with a combination of MDP and clindamycin when compared to animals receiving placebo or either agent alone, indicating that immunomodulation might be beneficial in initially failing antibiotic treatment (*Lamont* et al., 1983). However, subcutaneous MDP pretreatment failed to enhance cytotoxic activity of the MPS towards intravenous infection with *E. coli*, which is most likely due to the intravenous route of infection (*Dunn* and *Horton*, 1990). Although good results are obtained with prophylactic administered MDP, mice

infected intramuscularly could even be protected by intravenous administration of MDP 1 h after infection (*Chedid* et al., 1977).

As stated before, due to the lack of sufficient host defence, especially immunocompromised patients are prone to severe infections, with often a bad prognosis. Important therefore is the potency of the immunomodulators to enhance host resistance adequately in the immunocompromised host. MDP was shown to enhance survival from subcutaneous infection with *K. pneumoniae* in 7-day old new-born mice, this in contradiction with LPS treatment (*Parant* et al., 1978). The results indicate that MDP does not only affect the macrophages directly, but must also have other activities, which are absent in LPS. It is claimed by the same authors that MDP is capable of enhancing the host defence to a *K. pneumoniae* infection in thymectomised, irradiated, and bone marrow reconstituted mice (*Parant* et al., 1976). Galland, Polk and colleagues showed that *K. pneumoniae* infected wounds can be treated to some extent with MDP in immunocompromised mice (*Galand* et al., 1983a; 1983b; *Galland* and *Polk*, 1982; *Polk* et al., 1982; 1990). Survival in mice starved for 48 h and treated prophylactically with 500 μg MDP before intramuscular infection with *K. pneumoniae* was increased to approximately 90% compared with 40% in the controls (*Galland* et al., 1983a). The MDP treatment resulted also in lower local and systemic bacterial spread and increased survival in mice immunosuppressed by cyclophosphamide (*Galland* et al., 1983b). However, immunosuppression with hydrocortisone was shown to have a deleterious effect in this wound infection model on the host defence following activation by MDP. Administration of MDP prior to inoculation of burn wound with *P. aeruginosa* had no beneficial effect on survival in mice (*Stinnett* et al., 1983). These results indicate that although host defence can be augmented towards infections in immunocompetent hosts, less favourable effects are observed in the immunocompromised host.

Hershman and colleagues (1988a) demonstrated that administration of IFN-γ can augment the host resistance to a *K. pneumoniae* infection. Mice infected intraperitoneally with *E. coli* after which the mice were secondarily infected intramuscularly with *K. pneumoniae* received a daily subcutaneous dose of 7500 U IFN-γ for 6 days experienced a 2-fold increased survival compared with the controls (63% and 35%, respectively). Prophylactic administration of IFN-γ to mice receiving *K. pneumoniae* intramuscular or as a wound infection resulted in significant increase in survival compared with the controls (*Hershman* et al., 1988a; 1988b; 1988c; 1989). In this model also therapeutic administered IFN-γ (1 h after intramuscular challenge) resulted in significant augmented host defence (*Hershman*, 1989). However no beneficial effect was seen when these mice were infected with *P. aeruginosa* (*Stinnett* et al., 1983). Mice infected in the right hind leg and receiving IFN-γ subcutaneously in the same leg showed the same improved survival compared with mice treated with IFN-γ in the other leg, indicating a systemic activity of the host defence by IFN-γ (*Hershman* et al., 1988b). These results indicate that host defence activation by IFN-γ is probably mediated by macrophages not necessary located at the site of infection, but resulting from a general strengthening of the host defence.

LIPOSOMAL MTPPE AND INTERFERON-γ

Although good macrophage activation and antimicrobial activity is shown after exposure to immunomodulators *in vitro*, *in vivo* results are often quite disappointing. Actually this is not surprising. The *in vivo* experiments are complicated by several factors, of which next to short half life, dilution, and lack of significant localisation at site of interest are the most important.

Important advantages of the use of liposomes as carriers is that by encapsulating of the agents in liposomes half life in the body is prolonged, high concentrations at specific sites can be reached, co-encapsulation of agents facilitates synergy *in vivo*, toxicity is reduced, an immunological reaction is prevented. Liposomes are microscopic vesicles consisting of one or more lipid bilayers surrounding an internal aqueous compartment. Liposomes are biodegradable, and non-immunogenic when composed of natural phospholipids. A variety of agents can be entrapped in liposomes: hydrophobic agents with high efficiency in the lipid bilayers, and hydrophilic agents in the inner aqueous space. As the macrophage is believed to be the most important target cell for immunomodulation the use of classical liposomes (ranging from 1 to 20 μm in diameter), which have a natural fate to localise in large numbers in these cells, is quite obvious.

The advantage of classical liposomes to rapidly accumulate in MPS cells, primarily the macrophages residing in the liver and spleen (*Melissen* et al., 1994a), results in an augmented localisation of the encapsulated agent in the macrophage. In mice LE-MTPPE was preferentially taken up by the liver and spleen (32% and 17% respectively after 60 min). Localisation in the lung however only reached 8.4% of the injected dose after 5 min, declining rapidly below 5% after 60 min (*Melissen* et al., 1994a).

Initial studies with liposomes of PC and PS (molar ratio, 7:3) encapsulating MDP demonstrated that MDP was poorly retained within liposomes after their preparation (50% released in 5 h at 37°C) (*Phillips* and *Chedid*, 1988). The lipophilic derivative MTPPE however, has been shown to associate more efficiently with the liposome (93%), and is also much more stable (*Dukor* and *Schumann*, 1987; *Gay* et al., 1993). The encapsulation of MTPPE and IFN-γ prolongs half life of these agents in the body. They are not excreted shortly after intravenous administration (*Fogler* and *Fidler*, 1984). The plasma levels of free MTPPE is very low when liposome-encapsulated (*Gay* et al., 1993). There was also no macrophage mediated release. The rapid clearance of the LE-MTPPE from the circulation is mediated by the tissue macrophages, and not via excretion in the urine. Intact liposomes can be observed in macrophages for several days (*Fidler* et al., 1988; *Raz* et al., 1981). These results indicate that LE-MTPPE forms a depot of immunomodulatory material within the macrophage and considerable time (up to days) is necessary to degrade the liposome to release the incorporated muramyl peptide.

The successful use of IFN-γ for *in vivo* immunotherapy is also limited by the rapid clearance of the cytokine from circulation, and the potential toxicity from high dosage regimens (*Goldbach* et al., 1995; *Bennet* et al., 1986; *Kurzrock* et al., 1985). Free IFN-γ has a serum half life of approximately 20 min, and is degraded and secreted from the body. Liposome-encapsulation of IFN-γ (LE-IFN-γ) increases half-life and the ability of this agent to stimulate the host defence.

The increased activity of LE-MTPPE and LE-IFN-γ over free immunomodulators was shown in an *in vivo* infection model using *Listeria monocytogenes* in our laboratory. Encapsulation of MTPPE or IFN-γ increased their efficacy 33- and 66-fold respectively in mice infected with *L. monocytogenes* (*Melissen* et al., 1993). An increased activity of LE-MTPPE over free MTPPE was also shown in a *K. pneumoniae* infection model (*Melissen* et al., 1994b). Moreover, MDP encapsulated in liposomes was 10- to 15-fold more active to *S. typhimurium* and *S. enteritidis* infection as free MDP (*Phillips* and *Chedid*; 1987).

Both MTPPE and IFN-γ induce unwanted side-effects such as fever, weight loss, liver and kidney toxicity, and MTPPE induces also histopathological changes in arteries. Liposomal encapsulation decreased toxicity of MTPPE, the liposomal formulation had a no-toxic-level of 0.1 mg/kg compared with 0.01 mg/kg for free MTPPE (*Schumann* et al., 1989). Reduction of toxicity of IFN-γ by liposomal encapsulation has also been demonstrated (*Hockertz* et al., 1991).

Together, these results demonstrate that liposomal encapsulation reduces toxicity of the agents by shielding them of from the body, and reducing localisation to sensitive sites (site avoiding delivery), but also increases localisation at the site of interest :the macrophage (site specific delivery). This means that lower dosages can be applied, and better results obtained *in vivo*. However, it was shown in our laboratory that LE-MTPPE administered after infection with *K. pneumoniae* could also have a dose depending negative effect on the host resistance (*Melissen* et al., 1994b). Most important observation is that in the *K. pneumoniae* infection models best antimicrobial effects were obtained when immunomodulators were administered 24 h or more before infection (*ten Hagen* et al., 1995; *Parant* and *Chedid*, 1985; *Melissen* et al., 1994b).

The possibility of co-encapsulation of agents into liposomes also provides an important tool for drug delivery *in vivo*. *In vivo* synergy is questionable since *in vivo* the simultaneous exposure of macrophages to additional immunomodulators after intravenous administration is expected to be minimal. With agents co-encapsulated in liposomes, simultaneous delivery of the agents to the macrophage is guaranteed. Synergy between MTPPE and IFN-γ in the free form was shown *in vitro* using *L. monocytogenes* infected peritoneal macrophages (*Melissen* et al., 1993). Co-encapsulation of MTPPE and IFN-γ also improved survival of mice suffering from a *K. pneumoniae* septicaemia compared with the agents in the free from (*ten Hagen* et al., 1995)

In a murine model mimicking a naturally acquired septicaemia with *K. pneumoniae* the effect of MTPPE and IFN-γ on the host defence was studied in our laboratory. In this model bacteria are injected intraperitoneally, allowing the bacteria to multiply and appear in the blood, resulting in a septicaemia followed by death of all animals within 5 days after challenge. A single prophylactic dose of 25 μg LE-MTPPE resulted in 30% survival (*ten Hagen* et al., 1995). However, repeated prophylactic administration of LE-MTPPE (5 dosages of 25 μg daily), resulted in a survival of 65%. These findings indicate that the MPS cells do not become refractory to treatment. The beneficial effect of multiple treatment was also shown with an MDP analogue in an intraperitoneal infection model with *P. aeruginosa*: increased survival form 45% in control mice or mice treated with a single dose of norMDP, to 90 % in mice receiving 4 dosages (*Fraser-Smith* and *Matthews*, 1981).

In the *K. pneumoniae* septicaemia model utilised in our laboratory also the effect of LE-IFN-γ and the liposome-encapsulated combination of IFN-γ with MTPPE was studied. Intravenous injection of a single dose of LE-IFN-γ 24 h before infection resulted in 15% survival (*ten Hagen* et al., 1995) whereas five dosages of LE-IFN-γ could further increase the survival of mice to 65%. Moreover, combination of MTPPE together with IFN-γ by co-encapsulation in liposomes resulted in 100% survival (*ten Hagen* et al., 1995).

A VIEW ON THE MECHANISM

From the discussed studies it can be concluded that muramyl peptides and IFN-γ are potent stimulators of macrophage function. Exposure of macrophages to these agents results in an enhanced metabolic activity, excretion of ROI and RNI, production of important host defence activating monokines, and an increased antimicrobial activity of the cells. However, *in vitro* studies also frequently show that only macrophage activation is not sufficient.

Studies with macrophages exposed to LE-MTPPE, LE-IFN-γ or LE-MTPPE/IFN-γ *in vitro* demonstrated an enhanced production of nitrogen or oxygen intermediates when stimulated with heat-killed Gram-negative bacteria (*ten Hagen* et al., 1995). However, an increased antibacterial activity to *K. pneumoniae* could not be found *in vitro* when isolated macrophages were exposed to the above mentioned immunomodulators. These results are very striking as macrophages are thought to be the primary target for the immunomodulatory agents, certainly when encapsulated into liposomes. Moreover, the *in vitro* results also indicate that the observed increase in survival in immunomodulator treated mice suffering from a *K. pneumoniae* septicaemia, can not be explained solely by the increased activity of the tissue macrophages themselves.

It has been demonstrated that administration of MDP or MTPPE (free or liposome encapsulated) resulted in an increased blood clearance capacity of the MPS cells (*Ausobsky* et al., 1984; *Melissen* et al., 1992; *Parant* et al., 1978; *Fraser-Smith* et al., 1982; *Izbicki* et al., 1991). It was therefore speculated that increased survival from Gram-negative infection in mice induced by these immunomodulators resulted in the first place from an augmented phagocytic activity of the tissue macrophage, and hence an increased clearance of bacteria from blood. Studies with IFN-γ also demonstrated host defence activation, which was speculated to be a result of increased bacterial clearance rather than prevention of systemic spread (*Izadkhah* et al., 1980; *Matsumara* et al., 1990).

Others demonstrated that also locally the access of bacteria to the bloodstream is restricted in a wound infection after treatment with MDP (*Polk* et al., 1982). They claim that the MPS cells are not significantly enhanced by MDP, because increase of bacterial concentration in the liver coincided with an increase in the degree of bacteraemia. However, when bacteria do progress to the blood, for instance in an intraperitoneal infection model, lymphatic filtration can not explain the improved resistance after immunomodulator treatment. Increased phagocytic activity of the MPS cells on the other hand is also not a likely explanation as was shown *in vitro* as discussed above. These findings indicate that direct activation of macrophages by the immunomodulator is not the only

explanation for the increased host defence observed *in vivo* in severe (Gram-negative) infections. The improvement of the host defence *in vivo* might result from improved macrophage activity as well as enhanced macrophage cell number. Therefore increased clearance by the MPS might still be one of the explanations.

Melissen et al. (1994a) demonstrated that no correlation existed between liposome uptake and phagocytosis of bacteria *in vivo*. Therefore we propose that immunomodulation results in a cascade of events resulting in direct and indirect activation of macrophages, of which the indirectly activated macrophage may be the most active. Certainly in an intracellular infection the direct or indirect activation of macrophages would explain the observed increase in microorganism killing.

A finding which also might explain the host defence activation is the observed increase in the number of granulocytes and monocytes in the blood as was shown after treatment with free MTPPE or LE- MTPPE (*ten Hagen* et al., submitted for publication [a]; *Melissen* et al; 1992). We found that LE-MTPPE/IFN-γ treatment resulted in the first place in strongly augmented haemopoietic cell numbers in liver and spleen (*ten Hagen* et al., submitted for publication [a]). Especially myeloid cell numbers (monocytes and macrophages) were increased in these organs, whereas strongly increased erythropoiesis was also observed in the spleen. Secondly, treatment with LE-MTPPE/IFN-γ induced a shift in the bone marrow haemopoiesis towards generation of myeloid cells, whereas erythropoiesis declined. These results indicate that immunomodulation results in a dramatic increase in the number of MPS cells, resulting in an increased phagocytic capacity of this system. Together these results suggest that 1) increased recruitment of macrophages and granulocytes from bone marrow, 2) local proliferation of myeloid cells, and 3) augmented haemopoiesis in bone marrow account for the observed host defence improvement. Another striking observation is the dramatic augmented erythroblast cell number in the spleen after immunomodulation. It might be that the often observed anaemia accompanying sepsis is counteracted by the LE-MTPPE/lFN-y induced enhanced erythropoiesis in the spleen.

Upon stimulation macrophage produce many different cytokines (i.e. IL-1, TNF-α, IL-12 etc.). Especially IL-1 and IL-12 have a stimulating effect on T cells, and NK cells, responding with production of IFN-γ, which has in turn a stimulating effect on macrophages. Studies with MDP, MTPPE or IFN-γ demonstrated increased colony stimulating activity (colony stimulating factors (CSF) which stimulate cell proliferation) in serum after treatment. TNF-α production by macrophages is also strongly increased, and is known to have stimulating activities on growth and development of lymphoid tissues (*De Togni* et al., 1994). This mechanism also includes an important role for T cells. It was shown that potentiation of resistance by IFN-γ is possible in *Leishmania donovani* infected euthymic mice, but not in nude mice (*Murray* et al., 1995). Transfer of CD4+ or CD8+ T cells permitted nude mice to respond to IFN-γ treatment, which on the other hand could not be compensated with T cell derived cytokines alone. NK cells or NK derived endogenous IFN-γ did not seem to play any apparent role. The anti-Leishmanial effect correlated with a markedly enhanced mononuclear cell recruitment to infected liver foci. It was demonstrated in our laboratory that T cells play a very important role in the LE-MTPPE, LE-IFN-γ or LE-MTPPE/IFN-γ increased host defence

to *K. pneumoniae* septicaemia (*ten Hagen* et al., submitted for publication [b]). Depletion of CD4+ T cells or CD8+ T cells dramatically inhibited antimicrobial potentiation by the immunomodulators. Moreover, blocking of IFN-γ *in vivo* demonstrated that especially the production of endogenous IFN-γ is important in the host defence activation by the immunomodulators (*ten Hagen* et al., submitted for publication [b]). It was shown that treatment with LE-MTPPE/IFN-γ preferentially induced a Th1 T cell response in the spleen, resulting a high numbers of IFN-γ producing (Th1) cells. It is tempting to speculate that Th1 T cells, CD8+ cells and NK cells (cells known to produce IFN-γ) play a key role in the cytokine network induced by macrophage targeted immunomodulators.

PROSPECTS

With the ongoing problems with severe infections, and the inability of antibiotics to provide adequate therapy in immunodeficient patients, selected patient groups must be tested for the beneficial activities of immunomodulation. As is shown above the most promising results can be expected with prophylactic treatment. Especially patients who are prone to opportunistic infections, the immunocompromised patients, are of interest. Good results can only be anticipated when formulations are used in patients, still in possession of a good deal of their immune system, but on the brink of becoming severely immunosuppressed. In these patients combination of the best possible antibiotics with the most promising of the immunomodulators: in our perspective liposome-co-encapsulated MTPPE and IFN-γ, must be tested.

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