ADVANCES IN IMMUNOPHARMACOLOGY

3

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Contents

Special Lectures

From Natural Products Chemistry to Immunopharmacology

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ABSTRACT:

How a natural products chemist, armed with new analytical techniques, got interested in the biological properties of complex bacterial compounds and was led to the identification of new natural and synthetic immunomodulators.

KEY WORDS: Muramyl peptides; Mycobacteria; MDP: Chemistry of peptidoglycans; Trehalose esters; Cord factor; Cell wall skeleton; Wax D adjuvants; Sleep factors; Treatment of infections.

The lecture illustrates how the creation of new methods in organic chemistry led in the past fifty years to an explosive development of natural products chemistry and how in turn this gave the impetus to the discovery of natural immunomodulators which have been intensively discussed in this meeting.

I. New analytical methods

In the thirties two main techniques triggered the development: quantitative organic microanalysis by Boris Pregl in Graz (he received the Nobel Prize for chemistry in 1923) and corresponding laboratory methods of purifying, recrystallizing and distilling mg quantities of compounds (these micromethods had been mainly developed by Ernst Späth, professor of organic chemistry, Vienna University); then, in 1931 the renaissance of the chromatographic method of Michael Tswett, which had been almost forgotten at that time. In Heidelberg, Kuhn and Lederer (1931) separated the isomeric α- and β- carotenes as well as other carotenoids on a preparative scale by column chromatography, thus giving the impetus to the multifaceted diversification of chromatographic methods (see Lederer, 1972).

Invariably, chromatography of most previously described "pure" natural products gave several new substances.

II. From the Ascaris egg to immunodeterminant dideoxyhexoses

Our curiosity for natural products led us also into the field of invertebrate chemistry, which was at that time quite unexplored. In the fifties we analysed a peculiar lipid, coating the eggs of the parasitic worm Ascaris equi (discovered by E. Fauré-Frémiet in Paris in 1913 and called ascaryl alcohol). was in fact a mixture of several glycosides containing a new sugar, a 3,6-dide-oxyhexose, ascarylose (Fouquey, Lederer and Polonsky, 1957). We contacted O. Westphal and O. Lüderitz in Freiburg who had described isomeric

3,6-dideoxyhexoses as immunodeterminant end groups of endotoxins of gram-negative bacteria. In a joint synthetic effort the structure and stereochemistry of this new group of carbohydrates was cleared up (Fouquey, Lederer, Lüderitz, Polonsky, Stirm, Tinelli and Westphal, 1958). This was our first, quite unintentional, contact with immunochemistry.

III. An excursion into the mysterious world of Mycobacteria

A rich harvest of new compounds was then obtained in the fifties and sixties by a detailed study of the complex lipids of Mycobacteria (Lederer 1964, 1967, 1971).

Having established the criterion of chromatographic purity it was of course our aim to test the biological activity of chromatographically pure compounds, instead of the undefined mixtures tested previously.

Some of these, were the peptidolipids fortuitine (Barber, Jollès, Vilkas and Lederer, 1965) and peptidolipin NA (Barber, Wolstenholme, Guinand, Michel, Das and Lederer, 1966); the glycolipids mycoside A and B (Demarteau-Ginsburg and Lederer, 1963; Gastambide-Odier, Sarda and Lederer, 1965); or the mycosides C which are peptidoglycolipids (Chaput, Michel and Lederer, 1963; Vilkas, Rojas, Das, Wolstenholme and Lederer, 1966) are still awaiting detailed biological testing and might well give some interesting and unexpected results.

We had once suggested that the mycosides, "type-specific glycosides of mycobacteria" are "in charge of the public relations of the cell" (Lederer, 1967). And indeed, a recent discovery confirms this view. We had studied in detail the structure of the mycosides A and B (typical for M. kansasii and M. bovis, respectively) and characterized them as glycosides of a typical phenol-glycol esterified by two molecules of mycocerosic acid and carrying the carbohydrate on the phenolic OH group (Demarteau-Ginsburg and Lederer, 1963). Recently it was found by Hunter, Fujiwara and Brennan (1982) that one of the main antigens of M. leprae is a trisaccharide of this same esterified phenol-glycol; the trisaccharide can be used for the serodiagnosis of leprosy (Cho, Fujiwara, Hunter, Rea, Gelber and Brennan, 1984).

We also expect that the unique phosphatidyl-inositopentamannoside studied by Lee and Ballou, (1965) at Berkeley might have interesting immunological activities.

IV. Trehalose esters as immunomodulators

One of the glycolipids, "cord factor", discovered by Bloch in 1950 is an important natural immunomodulator. Its structure was established by Noll, Bloch, Asselineau and Lederer (1956) as a 6,6'-dimycolate of trehalose. The immunological properties of cord factor had been discovered by Bekierkunst (1968); independently Ribi, Meyer, Azuma, Parker and Brehmer (1975) isolated a biologically active glycolipid "P_3" which was shown to be identical to cord factor. Today natural and synthetic trehalose diesters are recognized as strong immunomodulators and, in particular, as activators of macrophages in vitro and in vivo (for reviews see Asselineau and Asselineau, 1978; Lederer, 1979; Goren, 1982; Lemaire, Tenu, Petit and Lederer, 1985).

TDM (trehalose dimycolate) can cure mice after one injection into an established fibrosarcoma and protects mice against various bacterial and parasitic infections. Lower homologues of TDM can be synthesized, but are generally less active (Parant, Audibert, Parant, Chedid, Soler, Polonsky and Lederer, 1978). More recently the use of aqueous suspensions of TDM has been developed by J.F. Petit at Orsay; these suspensions are very stable, much less toxic and as active as the TDM-oil preparations used previously, in particular for activating macrophages (Tenu, Lederer and Petit, 1980; Lepoivre, Tenu, Lemaire and Petit, 1982; Orbach-Arbouys, Tenu and Petit, 1983). These aqueous suspensions can protect mice

against infections by parasites such as Babesia microti (Clark 1979), Plasmodium berghei (Kumar, Ahmad and Lederer, 1984) and larvae of the Cestode Mesocestoides corti (White, Thompson and Penhale, personnal comm.), as well as rabbits against a lethal infection by Entamoeba histolytica (Sharma, Haq, Ahmad and Lederer, 1985).

V. From Wax D to MDP

One of the most complex peptidoglycolipids produced by Mycobacteria is the so-called Wax D which was first shown to be adjuvant active by Johns, Lederer and White, (1958). A detailed study of adjuvant active Wax D preparations led us to consider them as autolysis products of the cell wall and thus initiated a close study of the chemistry of the Mycobacterial cell wall (Lederer, 1971).

Mass spectrometry was an indispensable tool in establishing the precise structure of the repeating disaccharide unit of Mycobacterial cell walls, (Adam, Petit, Wietzerbin-Falszpan, Sinaÿ, Thomas and Lederer, 1969) was well as the structure of the tetrapeptide (Wietzerbin-Falszpan, Das, Azuma, Adam, Petit and Lederer, 1970).

Purified mycobacterial cell walls were adjuvant active as well as the monomer disaccharide terapeptide (Adam, Ciorbaru, Ellouz, Petit and Lederer, 1974), and also some smaller fragments, all containing muramic acid-L-alanine and D-glutamic acid. Finally, the first few mg of synthetic MDP produced by Merser, Sinaÿ and Adam (1975) were found to be fully active (Ellouz, Adam, Ciorbaru and Lederer, 1974).

We cannot dwell in detail here on MDP and the enormous literature which has been published since 1974 on chemical modifications and various favourable - and sometimes unfavourable-biological properties: these have been reviewed in detail elsewhere (Dukor, Tarcsay and Baschang, 1979; Parant, 1979; Adam, Petit, Lefrancier and Lederer, 1981; Lefrancier and Lederer, 1981; Lederer and Chedid, 1982; Kotani, Takada, Tsujimoto et al. 1982; Yamamura and Azuma, 1982; Adam and Lederer, 1984) and amply discussed in this Symposium.

VI. Desmuramyl peptides

A new chapter was opened by the discovery that a laurylated cell wall tetrapeptide (LTP) devoid of muramic acid was more or less comparable to MDP in its immunological properties (Migliore-Samour, Bouchaudon, Floc'h, Zerial, Ninet, Werner and Jollès, 1980). Japanese authors have described similar acyl peptides, all containing diamopimelic acid. In this series, at least the dipeptide D-glu-meso DAP seems to be the minimal essential structure (Kitaura, Nakagushi, Takeno, 1982; Izumi, Nakahara, Goto, 1983).

The lipophilic "desmuramyl" derivative L-alanyl-D-isoglutaminyl-L-alanyl-OCH_2-$CH(OH)$-CH_2-O-Mycolate ("triglymyc") is just as active as the corresponding muramyl derivative in stimulating non specific antibacterial resistance. It is adjuvant active in mice but inactive as an adjuvant in guinea pigs (Leclerc, Audibert, Chedid, Deriaud, Masihi and Lederer, 1984).

Mašek and Flegel (1983) have described an interesting new type of lipophilic desmuramyl peptide: L-ala-D-isoglin-adamantylamide.

Quite recently Parker, Migliore-Samour, Floc'h, Zerial, Werner, Jollès, Casaretto and Jollès (1984) have shown that a synthetic hexapeptide Val-Glu-Pro-Ile-Pro-Tyr corresponding to an enzymatically obtained fragment of human casein (residues 54-59) has very definite immunomodulating properties. It stimulates in vitro phagocytosis of SRBC by murine macrophages and increases in vivo the

resistance of mice to infection by K.pneumoniae. It has no chemical relation to cell wall peptides and opens thus quite new perspectives.

It is to be expected, however, that the activities of these various compounds are qualitatively different. Quantitative comparisons in various biological tests would certainly be useful.

Let us now highlight some of the recent facets of "MDP-ology".

VII. Specific targeting of muramylpeptides

A novel approach to specific targeting of muramyl peptides uses the property of "neoglycoproteins" (mannosylated proteins) to bind to a receptor at the macrophage cell surface and to be actively endocytosed. MDP bound to mannosyl-serumalbumin was shown to be a strong activator of mouse macrophages. Neoglycoprotein-bound MDP when injected i.v. or i.p. was found to activate alveolar macrophages leading to eradication of lung metastases (Monsigny, Roche and Bailly, 1984).

IgM monoclonal antibodies specific of mouse tumor cells coupled to MDP were shown to bind to relevant tumor cells and to induce activation of peritoneal mouse macrophages leading to 80% growth inhibition of target cells (Roche, Bailly, Midoux and Monsigny, 1984).

The activation of peritoneal exudate macrophages of mice to inhibit the in vitro proliferation of tumor target cells was obtained with very low concentrations of MDP conjugated to the synthetic polypeptide carrier poly-(DL-ala)-poly-(L-lys) (A--L) in presence of a monoclonal anti-MDP-antibody: this was explained by the fixation of the MDP-A--L antibody complex on Fc receptors of the macrophage membrane (Leclerc, Bahr and Chedid, 1984).

In this meeting Leclerc, Jolivet and Chedid (1985) have shown that MDP-mannose is specifically targeted to macrophages.

VIII. Muramylpeptides as sleep factors

A most exciting new chapter has been opened by the discovery that muramylpeptides are also "sleep factors" (Krueger, Pappenheimer and Karnovsky, 1982a). A concentrate isolated from human urine was found to contain muramic acid, alanine, glutamic acid and diaminopimelic acid. Synthetic MDP was then found active as well in prolonging slow wave sleep by intracerebroventricular infusion: MDP is also active when given i.v. and even per os (Krueger et al, 1982b).

The non-pyrogenic murabutide is not active as sleep factor; thus the simple shift of the amido group from the alpha to the gamma carboxylgroup of D-glu has a profound influence on the biological properties (Krueger, Walter, Karnovsky, Chedid, Choay, Lefrancier and Lederer, 1984).

More recently, a minor component of the urinary sleep factor was tentatively identified by fast atom bombardment mass spectrometry as the disaccharide tetrapeptide GlcNAc-MurNAc-L-ala-ν-D-glu-meso-DAP-D-ala; the major part was the corresponding anhydro compound (containing anhydromuramic acid) (Martin, Karnovsky, Krueger, Pappenheimer and Riemann, 1984). The anhydro compound could be formed during extraction by a bacterial transglycosylase, first described by Taylor, Das and Van Heijenoort (1975).

IX. "New activities of muramyl peptides"

MDP has been reported to have anti-inflammatory properties as shown by

experiments using classical models of acute inflammation (Zidek, Mašek and Sedivy, 1984). The mechanism of this activity remains to be explained. It might be related to the antioxidant effect of MDP (Yanev, Zidek, Kadiska, Serbinova, Mašek and Stoytchev, 1984).

MDP has also a protective effect (in vitro as well as in vivo) against administration of several hepatotoxic compounds (Mašek, Kadlecova, Kadlec, Zidek and Farghalli, 1984).

Some muramyl peptides have hypotensive properties. In search of inhibitors of the angiotensin converting enzyme (ACE) in cultures of Actinomyces, three "muraceins" were isolated from Nocardia orientalis and identified as muramyl peptides; muracein A, the most potent inhibitor, is MurNAc-L-ala-D-glu-meso-DAP-ε-amide, muracein B is a D-ala-D-ala derivative of muracein A and muracein C is a serine-containing muramylpeptide (Bush, Henry and Slusarchyk, 1984; Singh and Johnson, 1984).

X. A synthetic Freund's adjuvant

Let us now come back to Freund's adjuvant; we often wondered why only Mycobacteria and Nocardia asteroides could be used in this adjuvant; we thought this might be due to the fact that in other bacteria the peptidoglycans are masked by LPS, teichoic acids, etc. Goren (1982) has stressed, however, that in Mycobacteria the cell wall peptidoglycan carries a heavy load of mycolates, i.e. strongly lipophilic groups, amongst which TDM is an immunomodulator on its own right, as we have seen; and indeed, a synergism between MPD and TDM has been often described (for reviews see Lederer, 1980 a,b; Lederer and Chedid, 1982; see also Yarkoni, Lederer and Rapp, 1981; Masihi, Brehmer, Azuma, Lange and Muller, 1984).

So if we want to reconstitute a synthetic Freund's adjuvant, we would have to mix MDP with TDM, emulsify the two compounds with a pure hydrocarbon, for instance, with squalane or squalene and the perhaps even add some corpuscular material having a large surface; for instance aluminium hydroxide, which indeed has been used by Audibert, Przewlocki, Leclerc, Jolivet, Gras-Massé, Tartar and Chedid (1984) as a coadjuvant with murabutide for a hepatitis B vaccine.

And why not also add a trace of synthetic lipid A to this "immunococktail"?

XI. The future of muramylpeptides

In conclusion, one might wonder whether muramyl peptides will stand the test of time. Will they be used as adjuvants in vaccines, or as non-specific immunostimulants for prevention or cure of infectious diseases? Or in combination with chemotherapy? We trust that soon a positive answer will be given to one or the other of these questions.

Let us not forget, however, that muramylpeptides are omnipresent in our organism, that our blood is constantly receiving peptidoglycan fragments from the gut and that these fragments are our natural macrophage activators and sleep factors; they are natural and essential "vitamin-like" compounds (Lederer, 1982).

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