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HUMAN HAPTEN-SPECIFIC LYMPHOCYTES: BIOMARKERS OF ALLERGY IN MAN

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Environmental pollutants and other chemicals may have increasing impact on the immune system of human beings. Disregulation of the immune system by chemicals may be one of the reasons why the frequency of allergies and autoimmune diseases increases. Human hapten-specific memory lymphocytes can be detected in the blood of patients with drug-induced immunologic side effects but not in similarly exposed healthy individuals.

The immune reactivity of human lymphocytes in vitro *to white coloring agent—titanium dioxide (TiO_2), and mercurial conservatives thimerosal and phenylmercury—has been studied. It was found that out of 650 patients tested, 3% reacted to titanium dioxide. The percentages for phenylmercury and thimerosal were 14% and 7%, respectively. Human memory cells can be used as markers of susceptibility in future choices of appropriate additives in pharmaceutic products.*

Key Words: Haptens; Memory lymphocytes; MELISA; Titanium; Thimerosal; Drug allergy

INTRODUCTION

THE INDUSTRIAL REVOLUTION and the demand for a high standard of living contribute greatly to the increasing impact of environmental pollutants and other chemicals on the immune system of human beings (1). Disregulation of the immune system by chemicals may be one of the reasons why the prevalence of allergies and autoimmune diseases is increasing.

As regards pharmaceuticals, which are often used on a long-term basis, it is of great importance that these products are devoid of any potentially harmful additives which could induce unwanted side effects. Such side effects may be falsely blamed on the pharmaceutically active drug.

Modern immunology provides the tools for measurement of biological events which may occur in the body upon exposure to low molecular substances. Such biomarkers may be divided into three different categories: markers of exposure, markers of susceptibility, and markers of health effects (1,2).

BACKGROUND AND DISCUSSION

Human memory lymphocytes can be restimulated with various immunogenic substances such as proteins, viruses, or bacterial antigens *in vitro*. This so-called lymphocyte proliferation test (LTT, LST) has been used for years for the testing of cell-mediated immunity by clinical immunologists. In the immunotoxicologic laboratory, the test can be used

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for the detection of bacterial impurities in pharmaceutical preparations obtained with the help of molecular biotechnology. In this situation, bacterial antigen-specific human memory cells can be used as markers of exposure.

With regard to allergy induced by drugs, environmental chemicals or metals, it is generally accepted that human hapten-specific memory lymphocytes can be detected in the blood of patients with drug-induced immunologic side effects, but not in similarly exposed symptom-free individuals (1). Thus, specific memory cells can be used as markers of susceptibility in drug-treated patients and in occupationally exposed individuals. Since T lymphocytes participate in all forms of immunologic reactions, this test can detect both immediate and delayed-type hypersensitivity. Memory lymphocytes are generally long-lived and can be detected prior to the occurrence of the acute clinical reactions or, retrospectively, a long time after the sensitization event.

In the immunotoxicologic laboratory at Safety Assessment, the optimized lymphocyte proliferation test was originally developed for the detection of occupational allergy to penicillins, but also to other drugs such as alprenolol, chlormetiazole, quinidine, zimeldine, and terbutalin (3). During the recent studies it was recognized that even strongly irritative substances such as formaldehyde, Kathon CG, mercurials, or metoprolol epoxide may induce specific memory cells in allergic individuals and may therefore serve as markers of susceptibility (4,5,6).

With regard to metals, it is generally recognized that they may induce both immunomodulatory and antigen-specific responses when added to lymphocytes *in vitro* (7). To be able to use a lymphocyte proliferation test for the diagnosis of metal-induced allergies, it was, therefore, necessary to modify it in such a way that only antigen-specific responses were detectable. This was achieved by lowering the concentration of the metal used for lymphocyte stimulation *in vitro* (5, 6). The test was tentatively named MELISA (**M**emory **L**ymphocyte **I**mmunostimulation **A**ssay). The major methodologic differences as compared to a standard lymphocyte proliferation test are the use of defibrinated blood instead of anticoagulant-treated blood, the cultivation of lymphocytes in cultures of 1×10^6 cells instead of cultures of 2×10^5 cells, and a double reduction of adherent cells, at the start and at the end of cultivation. In addition, the use of inactivated human serum instead of fetal calf serum makes the test more sensitive by decreasing spontaneous proliferation in control cultures. With the help of MELISA, one can study the prevalency of *in vitro* responses to drug additives, such as pigments, preservatives, and antioxidants (Table 1).

During the years 1990–1995 the immunotoxicologic laboratory at Safety Assessment studied the activity of lymphocytes from patients with clinically verified or suspected metal intolerance to various metals used as components of dental metal alloys. In addition to metal pigments based on cadmium and lead, titanium dioxide (TiO_2), a white coloring agent, is used in many dental materials, such as composite fillings, dental cements, and root-fillings. An organic mercury preservative, phenylmercury, was previously used as a component of the root-filling material N_2, forbidden in Sweden since 1984. Also studied was the reactivity of lymphocytes to thimerosal, an ethylmercury based salt, widely used as a preservative in soft contact lenses, nose and eye drops, and in immunoglobulin-containing vaccines. In addition to metal intolerance, the patients suffered from

TABLE 1
Additives Which May Be Present in Drugs and Pharmaceutic Preparations

Pigments	Preservatives
Beta-Carotene (E 160)	Benzalconium chloride
Chinoline Yellow (E 104)	Benzoic acid (E 210)
Erythrosin (E127)	Citric acid
Iron oxide (E 172)	Methylparabene
Titanium dioxide (E 171)	Phenylmercury
	Thimerosal (Merthiolate)

multiple symptoms resembling Chronic Fatigue Syndrome (CFS), various skin and oral symptoms, and autoimmune diseases. The results are shown in Table 2.

The results indicate that significant proliferative response (SI > 5) to phenylmercury was obtained in 91 out of 661 patients tested (14%). Thirty-six patients out of 498 tested responded to thimerosal (7%). Twenty-two out of 649 patients tested (3%) responded to titanium *in vitro* with a stimulation index of more than five.

The corresponding values for stimulation index $>3 \leq 5$ were 28%, 11%, and 12%, respectively. The clinical significance of titanium-induced *in vitro* lymphocyte responses is unclear. Several patients with positive responses had titanium implants or reacted with side effects, such as gastrointestinal disturbances, following the ingestion of titanium-coated medicaments. The same pills without titanium coating were tolerated. Since TiO_2 is insoluble in water, the patch test, a standard test used by dermatologists, may be less suitable for evaluation of cellular immunity induced by titanium salts.

In a clinical setting, there is no doubt that thimerosal and phenylmercury may induce both local and systemic reactions (8–11). At the cellular level, thimerosal, as well as other organomercurials, affect Ca^{2+} homeostasis (12) and ethylmercury salts are exceedingly toxic to nerve tissue (13). Recently, it was demonstrated that ethylmercury is a major antigenic epitope in thimerosal hypersensitivity (14). The prevalence of thimerosal hypersensitivity based on patch testing varies among countries. An alarmingly high prevalence in young subjects has been reported from Scandinavia (15). Since many positive test reactions are not associated with a well-defined clinical picture, however, the clinical relevance remains, in many cases, unclear.

Titanium dioxide has been generously used in the pharmaceutical and food industries as a white coloring agent. Chemical properties of titanium are similar to other transition metals such as iron, copper, nickel, palladium, and manganese. Transition metals are unstable and bind strongly to protein groups. In surgery, titanium held its position within the hierarchy of biocompatibility in spite of frequently observed gross discoloration of tissue adjacent to implants (16,17). Human polymorphonuclear leucocytes are activated by TiO_2 whereupon they produce highly reactive oxygen metabolites (18), a fact that can explain recently described bactericidal properties of the pigment (19). The presence of TiO_2 in many dental creams may serve this purpose. Another report claims that among metals, titanium and nickel are the strongest modulators of bacterial strain sensitivity to antibiotics (20). Furthermore, Japanese researchers have reported changes in Ca^{2+} concentrations in the presence of TiO_2 ultrafine particles (21). Moreover, a photocatalytic effect of TiO_2 is presently of major interest in the field of breakdown of organic pollutants in water (22).

As far as biological effects are concerned, pulmonary alveolar proteinosis have been described in a painter, the finding confirming previous results from animal studies (23). In a Swedish study, strong inflammation has been observed in the skin adjacent to pene-

TABLE 2
Lymphocyte Responses to Phenylmercury, Thimerosal, and Titanium Dioxide

Antigen in culture	Tests with SI ≤ 3		Tests with SI > 3		Tests with SI > 5		
	#	%	#	%	#	%	Total
Phenyl-Hg	473	71.6	188	28.4	91	13.8	661
Thimerosal	443	89	55	11	36	7.2	497
TiO_2	572	88.1	77	11.9	22	3.4	649

1) Lymphocyte responses with stimulation indexes (SI) equal to or less than three are considered negative.

trating titanium implants (24). These and other reports appearing in the literature strongly question the "inertness" of titanium dioxide and its further use as a coloring agent in the pharmaceutical and food industries.

CONCLUSION

In conclusion, modern immunotoxicology offers powerful tools for the study of possible immunological and biochemical side effects of preservatives and pigments used in pharmaceutical products. At the individual level, there may be genetically vulnerable subjects reacting to certain drug additives with untoward side effects. In the future, the use of biomarkers such as specific memory cells may provide a clue for the choice of appropriate additives for use in pharmaceutical products.

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