Role of Metabolic Activation by Cytochrome P450s in Chemical-induced Immunosuppression

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Abstract

It is generally accepted that the immune system is one of the major target organs for many toxic chemicals. In addition, many toxic chemicals require metabolic activation by cytochrome P450s for their toxicity. Although the immune cells possess a limited amount of drug metabolizing capacity, metabolic activation of certain toxicants in liver and immune organs may have a significant role in immunosuppression. In the present studies, the possible role of metabolic activation by cytochrome P450s in chemical-induced immunosuppression was reviewed, with a particular emphasis on the methodological techniques to detect immunotoxicants requiring metabolic activation in vivo and in vitro. The immunosuppression induced by dimethylnitrosamine, cyclophosphamide, cocaine, 2-acetylaminofluorene and ethyl carbamate was described.

Liver is a critical organ for drug metabolism. Foods and drugs ingested into the body through gastrointestinal tract are absorbed to liver via hepatic portal vein and metabolized. Because liver possesses a strong activity of xenobiotic biotransformation and because many toxic chemicals require metabolic activation to exert their toxicity, the metabolites produced by liver would affect not only liver function but also other organs specifically targeted. In this viewpoint, the immune system that normally contains a very limited capacity for xenobiotic metabolism can be an important target for toxic chemicals requiring metabolic activation. To detect immunotoxic chemicals in vitro requiring metabolic activation, exogenous sources having xenobiotic-biotransforming activity would be essential (Holsapple et al., 1984; Yang et al., 1986).

The immune system is not normally considered as a primary organ involved in the absorption or excretion of drugs and chemicals. However, because immunocompetent cells require continued proliferation and differentiation for self-renewal, these cells would be expected to be sensitive to agents which affect stages of the cell cycle or the production of proteins necessary for these functions, such as lymphokines or cell surface receptors for growth factors. So any

disruption of the ability to recognize cell surface markers and antigenic determinants could damage the system. Although lymphocytes generally exhibit low metabolic capability themselves, metabolism by other tissues can activate inert xenobiotics and plays an important role in their effects on immunity. Immune system is also regulated by a number of other organ systems such as various nervous and endocrine systems, as well as the activity of liver. The consequences of direct or indirect interaction of immune system with these organs can be no changes in immune function, suppression of immunity, or immune enhancement (Luster et al., 1988; 1992).

Immune system is composed of a number of cell types that act in different ways. Because of this complexity, the strategies to identify immunosuppressive or immunostimulatory agents in laboratory animals are not simple. Therefore, tiered systems have been recommended. Although the testing panels vary depending upon the laboratories conducting the test and animal species employed, they include measures for the altered lymphoid organ weights and histology, quantitative changes in cellularity of lymphoid tissues, impairment of cell function at the effector or regulatory level, and increased susceptibility to infectious agents or transplantable tumors.

The guidelines recommended by the United States' National Toxicology Program for detecting immune alterations are composed of two tiered systems. In Tier 1 tests, it is intended to screen whether the testing material has an immunotoxic potential. The parameters for immunopathology include hematology, organ weight changes and histology which can be obtained from the general repeated dose toxicity testings. For humoral immunity, the IgM antibody response to T-dependent antigen and LPS mitogen response are recommended and, for cellular immunity, lymphoproliferation by T-cell mitogen and mixed leukocyte response to allogeneic leukocytes are recommended. For nonspecific immunity, natural killer cell activity is determined.

In the tier 2 comprehensive tests, all parameters to look at the mechanism of immunotoxic action by testing material are recommended. To determine the cellular target of toxic action, the quantitation of splenic B and T cell numbers are recommended using a flow cytometer. More advanced parameters are selected for testing humoral and cellular immune functions. The most important parameter in the tier 2 tests is the host resistance challenge model. There are syngeneic tumor models, as well as the challenge models of bacteria, virus and parasite. These models can mimic the in vivo situation that specific pathogenic antigen attacks the immune system.

The guidelines by Korean Ministry of Health and Welfares for detecting immune alterations are recommended only for new drug candidates. The antigenicity tests are recommended for a

long time due to an influence by the Japanese guidelines. Other functional tests have been included since 1994. But still the functional tests are not well established in Korea. For example, these functional tests are optional. So when one see a sign of immunotoxic potential of testing material in the repeated dose toxicity tests, then one can choose two parameters, one from cellular immunity tests and the other from humoral immunity tests. In addition, there is no such guideline for industrial chemicals other than drugs in Korea.

In vitro immunotoxicity testings with spleen cells have been used to detect immunotoxic chemicals, because spleen cells are able to respond certain antigens that added in cultures in an appropriate condition. However, a major pitfall is a limited xenobiotics-biotransforming activity of spleen cells. Therefore, it has been an issue to detect immunotoxic chemicals in vitro that require metabolic activation, such as dimethylnitrosamine, ethyl carbamate and cyclophosphamide (Jeong et al., 1995a; Tucker and Munson 1981; Yang et al., 1986). To solve this problem, conincubation of spleen cells with liver microsomes or primary hepatocyte cultures has been developed since past 10 or more years (Jeong et al., 1992; 1994a; 1994b; 1994c; Johnson et al., 1987, Kim et al., 1987; Tucker and Munson 1981; Yang et al., 1986). In the coincubation of spleen cells with liver microsomes, spleen cells are coincubated with liver microsome and testing chemicals for 0.5 or 1 hr and separated to culture with specific antigens like sheep red blood cells. Because the liver microsome does not contain phase II enzymes and because it is immunosuppressive when coincubated for a long time, the coincubation of spleen cells with primary hepatocyte cultures has subsequently been developed (Johnson et al., 1987; Kaminski et al., 1993; Kim et al., 1987; 1988; Yang et al., 1986). Because the hepatocytes are anchorage-dependent and spleen cells are anchorage-independent, two cells can be separated very easily following the coincubation. Therefore, after the coincubation, spleen cells are separated from hepatocytes for immunization with certain antigens in vitro for testing variable immune functions. Although there are many things to be optimized, this hepatocytes/splenocytes coculture system has been demonstrated to be an ideal method for detecting immunotoxic chemicals requiring metabolic activation.

The relationship between cytochrome P450s and immunosuppression can be studied in vivo. Prior to administration with testing chemicals, animals are pretreated with cytochrome P450 inducers to see whether the immunosuppression is potentiated compared to a vehicle-treated control. There are well-known inducers of cytochrome P450s, such as 3-methylcholanthrene and beta-naphthoflavone for cytochrome P450 1A, phenobarbital and beta-ionone for cytochrome P450 2B, ethanol and fasting for cytochrome P450 2E1 and dexamethasone for cytochrome P450 3A. However, only phenobarbital and beta-ionone could be useful for the purpose of investigating the role of metabolic activation in chemical-induced

immunosuppression, because other well-known inducers have been turned out immunosuppressive (Holsapple et al., 1993; Jeong et al., 1995b; 1995c; 1995d; 1996a; 1998; 1999; White et al., 1985). So it would be a matter how one can detect the immunotoxic chemicals requiring metabolic activation by cytochrome P450s 1A1, 2E1 and 3A1. Fortunately, however, pretreatment of animals with either phenobarbital or beta-ionone can induce not only cytochrome P450 2B but also other cytochrome P450s (Jeong et al., 1995c; Jeong et al., 1998).

It is one of our research goals to develop new model inducers of cytochrome P450s which may be able to induce one cytochrome P450 isozyme selectively without any toxicity in vivo at the dose for induction.

On the other hand, model inhibitors of cytochrome P450s are important equally in studying the possible role of metabolic activation in chemical-induced immunosuppression. One can confirm the role of metabolism in chemical-induced immunosuppression with specific cytochrome P450 inhibitors. For example, SKF 525-A, disulfiram and aminoacetonitrile have been introduced for these particular purposes, because they were relatively not immunosuppressive (Haggerty et al., 1988; Jeong et al., 1995a; Jeong et al., 1996b). In addition, a well-known inhibitor of monoamine oxidase, pargyline, has also been introduced as a cytochrome P450 inhibitor in the studies of dimethylnitrosamine-induced immunosuppression in the hepatocyte/splenocyte coculture system (Jeong et al., 1994b). For the same reason, it is one of our research goals to develop new model inhibitors of cytochrome P450s which may be able to inhibit one cytochrome P450 isozyme selectively without any toxicity in vivo at the dose for inhibition.

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