

Application of the popliteal lymph node assay (PLNA) for evaluation of the antigenicity of water-soluble food colors

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Abstract

The mouse popliteal lymph node assay (PLNA) has been proposed as an immunotoxicological test to predict the allergenicity of chemicals without additional adjuvant. Although a PLN response in the primary PLNA is also observed in association with non-specific activation induced by some irritants, the PLN response in the secondary PLNA in previously sensitized animals is used to detect memory immune responses without using adjuvant.

In this study PLNA was applied to evaluate the antigenicity of water-soluble food colors. The PLN cellularity index of an individual dye was calculated from the cell count of exposed and control PLNs of mice. The xanthene dyes Food Red No.3, Food Red No.104, and Food Red No.105, but not Food Red No.106 gave a high PLN cellularity index. The azo dyes and triphenylmethane dyes were not associated with any increase in PLN cellularity index. Indigo Carmine (Food Blue No.2) also had a high PLN cellularity index. The reactivity of the PLNA correlated well with the chemical structure category of the synthetic dyes. The high PLN cellularity index was considered to be associated with protein binding.

Key words: popliteal lymph node assay; PLNA; LLNA; synthetic dye; food additive

1. Introduction

The testing method for the antigenicity of food additives has not been fully established. Acute systemic anaphylaxis (ASA) and passive cutaneous anaphylaxis (PCA) have been used to test the antigenicity of medical supplies, but they are not suitable for detection of the antigenicity of low-molecular weight supplies. The local lymph node assay (LLNA) is widely used as an antigen detection method. In the LLNA an antigenic substance is applied to a mouse pinna, and the reactivity is observed. Since it is hard to retain water-soluble test solution at a mouse pinna, the improved method for applying LLNA to some water-soluble substances has been reported¹⁾. Recently the abdominal wall method (AW method) using the mouse abdominal wall and adjuvant has been used to detect anaphylactic reactions²⁾.

The popliteal lymph node assay (PLNA) was initially proposed as a method for detection of systemic autoimmune reactions³⁾, and later also as a method of assessing the immunostimulating and sensitizing potentials of drugs without additional adjuvant⁴⁾. Although a popliteal lymph node (PLN) response in the primary

PLNA is also observed in association with non-specific activation induced by some irritants, the PLN response in the secondary PLNA in previously sensitized animals is used to detect memory immune responses. Recently more than 130 compounds, including anti-arrhythmics, anti-convulsants, anti-depressants, non-steroidal anti-inflammatory drugs, cardiotropic drugs, antibiotics, and environmental chemicals, have been so far tested in one or more variations of the assay⁵⁾. A good correlation was found between the sensitizing potential of the chemicals tested and the occurrence of clinical reactions in humans⁵⁾, despite some discrepancies⁶⁾.

The present study was designed to evaluate the antigenicity of the synthetic dyes as water-soluble materials *in vitro* by the PLNA, since the synthetic dyes used as food additives are highly water-soluble. The relationship between the PLN cellularity index and the classification of the synthetic dyes was investigated.

In Japan, 83 synthetic dyes (tar dyes) have been approved for use as coloring agents in cosmetics, and Ordinance No.55 (December 13, 1972) of the Ministry of Health, Labour and Welfare classifies the synthetic dyes into three categories from the stand-