成果報告

題目:實驗性自體免疫前葡萄膜炎的"口耐受性"研究

Oral Tolerance and Experimental Autoimmune Anterior Uveitis

NSC89-2314-B002-249

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中文摘要

關鍵詞：黑色素、自體免疫、動物模型、葡萄膜炎口耐受性

葡萄膜炎一般認爲是一種自体免疫疾病，因反覆發生，造成角膜病變白內障青光眼或視網膜病變，而導致失明。其治療到目前爲止一直是以局部或口服腎上腺類固醇或其他免疫抑制劑如環孢靈素等爲主要的方法。這類療法因爲是廣泛性、無選擇性的免疫抑制，而有各種相當嚴重的副作用。

口耐受性是指口服一種抗原，身體對該抗原的反應方式會有所改變。以視網膜可溶性抗(S-Ag)爲例，在路易氏鼠口服S-Ag(或其它抗原爲對照組)數週後再於腳上注射S-Ag以引發實驗性自體免疫葡萄膜炎。發現口服S-Ag的一組實驗性自體免疫葡萄膜炎不會發作，而對照組口服其它不相關抗原則會如預期的發病，其抑制作用具抗原選擇性。最近因各種自體免疫疾病的動物模型的建立與應用，而能進一步探討口耐受性在治療與預防自體免疫疾病的可行性。有些疾病在動物模型與在小規模的人體試驗中獲得令人鼓舞的結果，如類風濕性關節炎、多發性硬節症、難纏的葡萄膜炎等。

本報告中使用口服可溶性黑色素相關抗原來防止實驗性自體免疫前葡萄膜炎。因實驗性自體免疫前葡萄膜炎在發病3-6週可幾乎完全復原，再次施打MAA時可引起實驗性自體免疫前葡萄膜炎再發，因此可以研究在初次發病前或發病後讓路易士鼠口服黑色素相關抗原，以研究口耐性初級預防實驗性自體免疫前葡萄膜炎發病或發病後次級預防實驗性自體免疫前葡萄膜炎再發的可能性。(而後者正是臨床上最希望能應用的。)結果是口服不可溶黑色素相關抗原無法抑制實驗性自體免疫前葡萄膜炎的發生與再發。這可能與本實驗中使用的是不可溶黑色素抗原，而黑色素本身即有促發發炎反應的作用有關。使用可溶性黑色素抗原於類似的實驗將是下一個實驗計劃的方向。

ENGLISH ABSTRACT

Keyword: Animal Model, Autoimmunity, Uveitis, Oral Tolerance

Uveitis is one of the leading causes of blindness. It was estimated that 10% of the blindness was caused by uveitis in USA. The treatment is mainly topical and systemic corticosteroid, and some of these patients may require the use of a variety of immunomodulatory, corticosteroid-sparing agents, such as cyclosporine or cytotoxic agents. To date, clinically oriented approaches have centered on the administration of pharmacologic substances that have a nonspecific effect on the immune response. The development of more effective treatment of organ-specific inflammatory disorders of putative autoimmune origin is an ongoing goal in many specialties of clinical medicine. Recently, alternative therapeutic strategies have been suggested based on our better understanding of immunologic mechanisms that lead to organ-specific inflammatory responses. The induction of immunologic tolerance, defined as a state of specific immunologic unresponsiveness to an antigen after exposure to that antigen,

is one such approach that has gained attention recently. One effective method of inducing immunologic tolerance is through the oral administration of antigen. The tolerance induced is called oral tolerance. One feature of oral tolerance is that "bystander" suppressive effect can be elicited to the organ or tissue that harboring the antigen. Oral tolerance has been tested in various animal models of autoimmune disorders, such as experimental autoimmune encephalomyelitis, collagen and adjuvant arthritis, experimental autoimmune diabetes, and experimental autoimmune uveitis. The effect of oral tolerance has also been tested in several clinical conditions in small scale with encouraging results, such as multiple sclerosis, rheumatoid arthritis, juvenile diabetes, Behcet's disease and other intractable uveites.

The most common disease entity of uveites in Taiwan is acute anterior uveitis (AAU). It is the recurrent nature of AAU that can result in blindness and socioeconomic loss though various complications, including glaucoma, cataract, and cystoid macular edema. Experimental autoimmune anterior uveitis (EAAU) has been established to simulate human AAU. It involves the use of melanin associated antigen extracted from bovine uveal tissue. In this report, we investigated the effect of oral tolerance in EAAU both as primary and secondary prevention, i.e. in unprimed and in primed animals. By feeding Lewis rats with insoluble melanin associated antigen the experimental autoimmune anterior uveitis was not suppressed. This unsuccessful induction of tolerance might be related to the use of insoluble melanin associated antigen, which is pro-inflammatoy itself. To repeat the whole experiment by using soluble MAA may well be the goal of next project.

MATERIAL and METHODS

Animals

Female and male Lewis rats will, 200-250 gm of body weight will be purchased from Experimental Animal Center, National Science Council, Taiwan.

Preparation of insoluble melanin associated antigen.

Fresh bovine eyeballs will be harvested and transferred to the lab within 3 hours of death. The iris tissue will be excised and minced with PBS, and filtered through gauze. The filtrate will be centrifuged at 10,000 g for 10 minutes. The pellet will be resuspended with PBS and treated with 2% SDS at 75℃ for 10 minutes and the insoluble part will be weighed , resuspended in PBS and stored at -70℃ for later use.

Immunizations of animals

100 μg insoluble MAA were resuspended in 0.1 ml of balanced salt solution (BSS). The emulsion was inoculated into footpad of Lewis rats. One week after injection, clinical signs of EAAU will be monitored daily with a slit-lamp biomicroscope for a period of 3 to 4 weeks. Upon onset of EAAU, the animals will be sacrificed and the eyes will be removed and processed for histopathologic evaluation

using 4% glutaraldehyde and 10% buffered formaldehyde as fixative.

Recurrence of EAAU

The same protocol as primary immunization will be done after complete recovery from primary EAAU, which usually takes 3-4 weeks.

Induction of oral tolerance

Lewis rats were fed a total of 20 mg melanin associated antigen and 40 mg of soybean trypsin inhibitor (STI; sigma, St Louis, MO, USA) administered in four feedings during 8 day period. STI (20 mg/ml) and MAA (5 mg/ml) was each dissolved or suspended in 0.15 mole/L sodium bicarbonate buffer (pH 8.0). Rats were deprived of food but not water for 12-18 h prior or each feeding of antigen. Rats will be gently anesthetized with ether and fed MAA (1 ml) and STI (0.5 ml) by gastric intubations. In some experiments, panels of rats received only MAA (1 ml) suspended in bicarbonate buffer and no STI. Control rats were given four feedings of STI in bicarbonate buffer (vehicle control) or nothing (non-fed control). Three days following the last feeding, animals will be injected with MAA plus CFA at footpad. These feedings will be done in unprimed rats (as primary prevention) or previously primed rats (as secondary prevention). Standard protocols for clinical examination and histopathologic examinations will be done to detect the occurrence and severity of EAAU.

RESULTS

1) The effect of antigen feeding to unprimed rats on EAAU (primary prevention)

Antigen ingestion	Incidence of EAAU	Severity of EAAU
MAA	4/5	3.5+
BSA	4/5	3.5+
Normal saline	4/5	3.5+

MAA: insoluble melanin associated antigen

BSA: bovine serum albumin

2) The effect of antigen feeding to sensitized rats on EAAU (secondary prevention)

Antigen ingestion	Incidence of EAAU	Severity of EAAU
MAA	3/5	3 +
BSA	3/5	3 +
Normal saline	3/5	3 +

MAA: insoluble melanin associated antigen

BSA: bovine serum albumin

DISCUSSION

Wells first described the phenomenon of oral tolerance in 1911. Oral tolerance is a long recognized method to induce peripheral immune tolerance. The primary mechanisms by which orally administered antigen induces tolerance are via the generation of active suppression or clonal anergy. Low doses of orally administered antigen favor active suppression whereas higher doses favor clonal anergy. The

regulatory cells that mediate active suppression act via the secretion of suppressive cytokines such as TGF β and IL-4 after being triggered by oral tolerogen. Furthermore, antigen that stimulates the gut-associated lymphoid tissue preferentially generates a Th2 type response. Because the regulatory cells generated following oral tolerization are triggered in an antigen-specific fashion but suppress in an antigen nonspecific fashion, that mediate "bystander suppression" when they encounter the fed autoantigen at the target organ. Thus it may not be necessary to identify the target autoantigen to suppress an organ-specific autoimmune disease via oral tolerance; it is necessary only to administer orally a protein capable of inducing regulatory cells that secrete suppressive cytokines at target organ. This was the reason why we chose to use melanin-associated antigen, which is still not a well-purified one.

However, feeding rats with insoluble MAA failed to suppress EAAU primarily and secondarily. This might be related to the pro-inflammatory nature of melanin pigment itself. The same effect has been noted in the induction of anterior chamber associated immune deviation (ACAID) with MAA. Soluble MAA, but not insoluble MAA, could induce ACAID and prevent EAAU primarily and secondarily. The use of soluble MAA in the same experiment design may well be the next project, though it will require much, much more bovine eyes to extract enough soluble MAA.

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