Bulletin of Faculty of Pharmacy, Cairo University xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Bulletin of Faculty of Pharmacy, Cairo University

journal homepage: www.sciencedirect.com

Review Paper

Experimental animal models used for evaluation of potential immunomodulators: A mini review

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ARTICLE INFO

Article history:
Received 26 June 2017
Accepted 26 August 2017
Available online xxxx

Keywords:
Immunomodulator
Animal model
Rodent
Fish
Cell line

ABSTRACT

Preclinical models of immunomodulatory studies are indispensable part in the process of drug discovery and development. Still, they are mimeo of human immunostimulation/suppression provided heterogeneity of latter. This review discusses various preclinical models of immunomodulatory studies viz. cell lines for immunomodulatory studies, murine models include humoral antibody (HA) response, delayed type hypersensitivity response, macrophage phagocytosis by carbon clearance method, effect on total leucocytes count, leucocyte mobilization studies, mice lethality test Fish models include phagocytosis by fish blood lymphocytes, specific and non-specific response in fish and intestinal bacterial colonization; whereas pathogen infestation model includes immunomodulation against *Eimeria vermiformis*.

A portfolio of these biological models has to be utilized strategically as the specific stage of process of drug discovery. During selection of such model it has to be kept in mind that the model must be physiologically relevant. At the same time, it should be able to aid in prediction of human response. Never the less, apart from being 'physiologically relevant', the decisive 'proof' regarding safety and efficacy of test drugs lies human studies. Still, sensible construal and envision of data resulting from these models to humans, and a conformingly more prominence placed on medical research during early stage of clinical trials, are therefore indispensable to mend on the clinical study rates for discovery of novel immunomodulatory agents.

Contents

Peer review under responsibility of Faculty of Pharmacy, Cairo University.

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http://dx.doi.org/10.1016/j.bfopcu.2017.08.002
1110-0931/

Please cite this article in press as: A. Ganeshpurkar, A.K. Saluja, Experimental animal models used for evaluation of potential immunomodulators: A mini review, Bulletin Facult Pharmacy Cairo Univ (2017), http://dx.doi.org/10.1016/j.bfopcu.2017.08.002

1. Introduction

The process of immunomodulation amends the immune system of an individual by prying with its usual functions. In case, when the activity of the immune system is enhanced, it is termed as immunostimulant effect which is observed by promotion in the activity of macrophages, granulocytes lymphocytes, etc.; whereas immunosuppression is observed by a diminution in confrontation against stress and infection. The process of immunostimulation and immunosuppression is desired to police up normal immunological functions. Hence, immunomodulatory agents are anticipated as novel alternatives for treatment of various immunological pathogenies like ulcerative colitis, asthma, allergy, arthritis [1–3].

Certain class of drugs like anti-allergics, corticosteroids, anticancer-chemotherapeutic agents, NSAIDs have been employed to control immunological emergencies and pathogenes. However, these drugs have many adverse effects. Phytoremedies seems to be significant sources as immunomodulatory agents. Ayurveda documents the role of 'Rasayana' as a 'system of rejuvenation' [4]. Therefore, it seems to be fruitful to explore phytoremedies for potential immunomodulatory activity.

There are numerous methods which are used to establish immunomodulatory effects of a test compound. However, the selection of suitable model appears to be intricate as each model has particular merits and demerits. Selection of a particular model is based on the hypothesis of the test and aim of study. *In vitro* and cell lines studies followed by *in vivo* studies ought to be carried out to derive precise molecular mechanism of immunomodulation. The present review focus to provide a comprehensive overview of various experimental models of immunomodulatory research.

2. Models for immunomodulatory studies

Laboratory animals are inexorable part of biomedical research. They are considered as one of the best models of diseases including cancer, toxicology and neurological studies [5]. They seem to be the best substitutes of human beings for experimental research. Albino wistar rats and Sprague-Dawley rats are used in immunomodulatory studies [6,7]. BALB/c mice [8], Swiss albino mice [9], Dunkin-Hartley guinea pigs [10], *Cyprinus carpio* (fish) [11] have been used as model in immunomodulatory studies

3. Cell lines and immunomodulatory studies

Immortalized cell line comprises of the populace of cells derived from a multicellular organism that in general not flourished infinite, however, owing to mutation, have dodged typical cellular senectitude to continue division. Various cell lines used in immunomodulatory studies have been summarized in Table 1.

Table 1
Cell lines used in immunomodulatory studies.

Cell lines	Mechanism observed during immunomodulation
K562cell line	Stimulation of NK cells activity against K562 cells
J 779 macrophage cell line	Inhibition of chromate-induced cytotoxicity
K562 cell line	Decreased production of NO
Cutaneous squamous cell carcinoma cell line	Increased cytotoxic T lymphocytes

4. Murine models

Modulation of the immune system may be either cellular or humoral. There are various models used in immunomodulatory studies. Criteria for choice of the suitable design seem to be difficult as each model is relevant to a different type of immune challenge condition. Various models used experimentally to evaluate immunomodulatory effects are:

4.1. Humoral antibody (HA) response

Humoral response is demonstrated by antibody molecules which are products of 'B lymphocytes' and 'plasma cells'. Immunoglobulins (Ig) like IgG and IgM are chief immunoglobulins which are associated with opsonization, complement activation, and neutralization of toxin [12–14]. Humoral antibody response is studied by injecting prepared erythrocytes of sheep. Antigen-antibody reaction is observed in various types of immune responses. After exposure to antigens, the antigen-specific immune response is observed as a result of expression of antigen-presenting cells, B-cells and T helper cells which leads to the production of Ig M.

To prepare sheep red blood cells (SRBC), sheep blood is collected in Alsever's solution, and cells are isolated by centrifugation at 1000 rpm for 15 min. Plasma and the buffy coat is removed followed by washing of cells with five volumes of 0.9% NaCl thrice. The so formed pellet is suspended in two volumes of 0.05 M Tris-HCl with 0.1 mM EDTA (pH 7.6) and mixed thoroughly and again centrifuged at 25,000*g* for 30 min. The process may be repeated till the supernatant becomes clear. The pellet is resuspended in 0.1% sodium dodecyl sulfate (SDS) with 0.02% sodium azide. Finally, the membrane antigens are dialyzed against 0.1% SDS in and stored at −20 °C [15]. Protein content is estimated by Bradford assay [16].

To study humoral antibody response, animals can be divided suitability into several groups. Animals are immunized with SRBC (0.1 mL, 25% suspension in saline). On exposure to such antigenic substance, antibody formation is promoted by alpha globulin antibodies. The antibody production is also stimulated by administration of exogenous substances [17,18]. When animals are sensitized by SRBC, antigen diffuses in the extravascular space and enters the 'lymph node' which is taken up by macrophages that line up the lymph tissues. Antigenic peptides get combined with 'MHC class II molecules' and finally produce a significant number of daughter cells, some of which serve as memory cells while others get converted to 'plasma cells' that produce a huge amount of antibodies [14,19]. After about two and three weeks, blood from animals is collected and processed to obtain serum. The antibody titer is determined by using microtitre plates is such a way that the subsequent wells get twofold dilutions of the antibodies present in the serum (Fig. 1). To it, SRBCs are added observed for hemagglutination. Highest dilution is giving hemagglutination taken as the antibody titer [20]. Antibody titer obtained in the second and third

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