

## 29.2 Cellular immunology

cells were sensitive to treatment with anti-theta whereas the hapten-primed cells were not [6]. On the other hand it has been shown that the hapten-primed cells are sensitive to anti-MBLA and not to the carrier-primed cells [7, 8].

In this way the carrier-effect in mice has been shown to be the result of an act of antigen-mediated cellular co-operation between at least two types of lymphocytes. One is derived from the thymus and the other from the bone marrow. The thymus-derived or T lymphocytes require an intact thymus for maturation [9, 10] and are found in varying proportions in the peripheral lymphoid tissue (see Table 29.1 for organ distribution [7, 11]). The thymus-derived

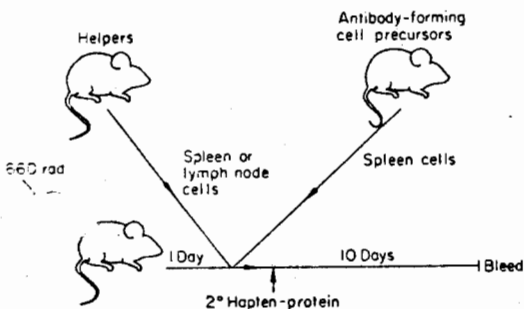


FIG. 29.1. Diagram of the cell transfer system.

lymphocyte does not secrete any detectable antibody [12] but is involved in both the humoral and cell-mediated immune responses [9]. It is a helper cell. The bone marrow-derived or B lymphocyte develops independently of the thymus. The B lymphocyte is the antibody forming cell precursor (AFCP) [13]. The thymus-derived lymphocyte, helper cell, binds antigen by means of a carrier determinant and presents the hapten determinant to a bone marrow-derived lymphocyte, AFCP, which is then somehow triggered to produce antibody.

This implies that for a molecule to be immunogenic it must possess at least two antigenic determinants [14]. One determinant acts as the carrier and the other determinant then acts as the 'hapten equivalent'.

### Reagents and equipment

Tissue culture medium will suffice as a salt solution to make the cell suspensions. Care must be taken to ensure that the medium does not contain any protein which will cross-react with the antigens being

used. Two very good solutions that contain no protein and are inexpensively made in the laboratory are: Gey's salt solution and Balanced Salt Solution (Hanks). See the appendix to Chapter 27 of this text for the formula for Gey's salt solution.

### Balanced salt solution (BSS)

#### Stock I

10.00 g Dextrose  
0.06 g  $\text{KH}_2\text{PO}_4$   
(or 3.58 g  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ )  
1.89 g  $\text{Na}_2\text{HPO}_4$   
0.10 g phenol red

Make up to 1 litre with double-distilled water

TABLE 29.1. Tissue distribution of thymus derived and bone-marrow derived lymphocytes in the mouse

Tissue	Thymus derived (%)	Bone marrow derived (%)
Thymus	100	0
Thoracic duct lymphocytes	80-90	10-20
Lymph node	60-85	25-35
Spleen	25-45	50-60
Bone marrow	0	~20

The above values are estimates derived from data published by Raff (1971) and Niederhuber & Möller (1971) using anti-theta and anti-MBLA antisera.

#### Stock II

1.86 g  $\text{CaCl}_2$   
4.00 g KCl  
80.0 g NaCl  
2.00 g  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$   
2.00 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

Make up to 1 litre with double-distilled water

Dilute 100 ml of Stock I to about 800 ml with double-distilled water, then add 100 ml of Stock II and bring the volume up to 1 litre.

### Proteins

CGG: Chicken  $\gamma$ -globulin, prepared from fresh chicken serum by fractionation with 45 per cent saturated ammonium sulphate.

BSA: Bovine serum albumin, purchased from

Armour, Cohn fraction BSA is used for the ser

HSA: Human serum Behringwerke ('reinst')

OA: Egg albumin, p

MGG: Mouse  $\gamma$ -globulin, purified by ion exchange chrom

### Haptens

DNP: For serology, solutions of the haptens are amino-lysine- $\alpha$ -amino-2,4,6-trinitrophenyl (DNP), prepared by the method of Mitchison, Pitt-Rivers & Hunter (1957) using  $^{125}\text{I}$ , according to the method of Hunter & Greenwood (1962).

TABLE 29.2. Physicochemical response

Hapten	$\lambda_{\text{max}}$
NIP	430
DNP	360
OX	360

iodinated product, 4-amino-lysine- $\alpha$ -amino-2,4,6-trinitrophenyl (DNP) is separated from the reaction on a Sephadex C column contained in the first fraction. The  $^{125}\text{I}$ -DNP-lys-DNP in the first fraction is stored in borate buffer, about  $3 \times 10^{-7}$  M.

NIP: 4-Hydroxy-3-nitrocaproic acid (NP-CAP), prepared by the method of Brownstone & Hunter (1962) with  $^{125}\text{I}$  by the method of Hunter & Greenwood [16], yielding 4-hydroxy-3-nitro-L-lysine- $\alpha$ -amino caproic acid (NIP) which is separated from N $^{125}\text{I}$  by passing from an ion-exchange resin column. It is applied to a small column (1 ml syringe) of Dowex, 1, washed with distilled water and differentially eluted with glacial acetic acid. The