THE REQUIREMENT FOR C3 RECEPTORS ON
THE PRECURSORS OF
19S AND 7S ANTIBODY-FORMING CELLS

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The function of receptors for C3 on B lymphocytes is not known (1); nor is it
certain whether the initiation of either 19S or 7S antibody production requires
the presence of complement receptor lymphocytes (CRL). In a previous publication
from this laboratory it was reported that the removal of CRL from an
immune population of lymphocytes severely reduced but never eliminated an
adoptive 7S anti-2,4-dinitrophenyl (DNP) response in heavily irradiated recipi-
ents (2), suggesting that the precursors of 7S antibody-forming cells were
present not only in the CR+ but also the CR− subpopulations. Doubts about the
interpretation of these experiments centered on the degree to which the CRL-
depleted populations were still contaminated with small numbers of CR+ cells,
on the contribution to the response by radioresistant 7S precursor cells of
recipient origin, and on the possibility that the triggering of CR− cells may have
been an artifact of an allogeneic environment (3). The activity of radioresistant
precursors also prevented any conclusion about the role of CRL in 19S responses.
In view of these uncertainties and the recent speculations about the function of
C3 receptors (1, 4-8), the requirement for CRL in the induction of 19S and 7S
antibody production has been reinvestigated.

Materials and Methods

Animals. Inbred HO (hooded Ag-B5) rats were used as donors of TDL. Recipients were either
rats of the same strain or F hybrids between HO and DA (agouti, Ag-B4).

Irradiation. Recipient rats were given 850 rad from either a 60Co source at 35 rad/min or from a
137Cs source at 120 rad/min 24 h before cell transfer, except where stated otherwise. Results were
not affected by the type of radiation source. Irradiated rats received terramycin, 9 g/liter, in their
drinking water from the day of irradiation.

Antigens and Immunization. Bovine gamma globulin (BGG) and human serum albumin
(HSA) were dinitrophenylated by the method of Little and Eisen (9). Degrees of conjugation were
estimated by OD measurements at 280 nm and 360 nm using an Em for DNP of 17,400.

Donors were immunized intraperitoneally with 1 mg of alum-precipitated antigen together
with 2 × 106 killed Bordetella pertussis organisms (The Wellcome Research Laboratories, Becken-

1 Abbreviations used in this paper: AFCP, antibody-forming cell precursor; BGG, bovine
gamma globulin; CRL, complement receptor lymphocytes; EAC, erythrocyte-antibody-comple-
ment complex; FcRL, Fc receptor lymphocytes; FCS, fetal calf serum; FyG, fowl gamma globulin;
HSA, human serum albumin; IgL, immunoglobulin-bearing lymphocytes; PFC, plaque-forming
cells; TDL, thoracic duct lymphocytes.
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ham, England). Recipients received 1 mg of antigen in Dulbecco's A+B (DAB/10% fetal calf serum [FCS]) intravenously at the time of cell transfer.

Cells. Cells were collected from the thoracic duct into 5 ml DAB (Oxoid, London, England) containing 20 U/ml of heparin. Collections were made either for 6 h at room temperature or for 12 h on ice. Cells were washed twice in DAB/10% FCS before further manipulation and their viability always exceeded 95% as judged by trypan blue exclusion. Rats immunized with antigen 7-16 wk previously were used as a source of primed thoracic duct lymphocytes (TDL). Fractionation of cells according to cell diameter was kindly carried out by Dr. S. V. Hunt, Medical Research Council, Cellular Immunology Unit, Sir William Dunn School of Pathology, Oxford University, Oxford, England, using the 1 g sedimentation method described by Hunt et al. (11).

[3H]Thymidine Labeling of Lymphocytes In Vivo. HO rats were given 100 μCi [3H]thymidine (sp act, 5 Ci/mmol; Radiochemical Centre, Amersham, England) intravenously immediately after cannulation and the same dose 8 h later. 12 h after the second injection TDL were collected for 6 h. This regime labels the majority of large lymphocytes in TDL but very few of the small (10). Scintillation counting of labeled TDL was carried out as described by Howard et al. (12).

Plaque-Forming Cell (PFC) Assays. Direct and indirect (enhanced) PFC in single cell suspensions of spleen were assayed by the method of Cunningham and Szenberg (13) as described by Parish and Hayward (14). Target sheep erythrocytes (SRBC) were sensitized for lysis by anti-DNP antibody and guinea pig complement (C) by coating them with DNP-conjugated anti-SRBC fowl gamma globulin (FyG). A conjugation ratio of eight was used throughout. Indirect PFC were revealed by the addition of 10 μl of an optimum dilution of rabbit-antirat γ/2γ, kindly provided by Dr. A. F. Williams, Medical Research Council, Immunochemistry Unit, Department of Biochemistry, Oxford University, Oxford, England. Assays were performed 7 days after cell transfer. The donor or recipient origin of PFC was determined by means of cytotoxic alloantisera (2).

Preparation and Depletion of Fc Receptor Lymphocytes (FcRL), CRL, and Immunoglobulin-Bearing Lymphocytes (IgL). Rosettes between SRBC and FcRL, CRL, and IgL were prepared as described by Parish and Hayward (14) except that in the preparation of erythrocyte-antibody-complement complexes (EAC) C5-deficient serum (final concentration 1:6) from DBA/2 or AKR mice was used. These sera produced no hemolysis when incubated with antibody-coated SRBC. In contrast, C-induced hemolysis with normal mouse serum prohibited its use at concentrations in excess of 1:20. Such concentrations are less than optimal for the preparation of EAC (15).

In the preparation of rabbit Fabγ-antirat Fab-coated SRBC for rosetting with IgL it was found essential to use SRBC that were completely resistant to hemolysis when washed in normal saline. Possibly, free hemoglobin competes with the anti-Fab reagent for the chromic chloride used to couple the latter to the SRBC. Rosettes and total lymphocytes were counted simultaneously after adding to the suitably diluted rosette suspension an equal volume of Gentian Violet (E. Duvall, personal communication). Rosetted and nonrosetted lymphocytes were separated by sedimentation through a discontinuous density gradient produced by a mixture of Isopaque and Ficoll (14).

Results

Effect of CRL Depletion of TDL on the Adoptive Direct and Indirect PFC Response in Parent to F1 Cell Transfers. In studying the performance of B cells in transfer experiments it is essential to know that the antibody-forming cells which arise in the recipient are derived from the donor's lymphocytes. Parish and Hayward (2) on the one hand, showed that 850 rad was not sufficient to eliminate a host contribution to both direct and indirect PFC response to DNP when F1 TDL from primed donors were transferred to parental strain recipients. On the other hand, C. R. Parish (personal communication) found no such contribution from the irradiated host in parent to F1 transfers and this design was used in the present experiments on the role of CRL in the adoptive anti-DNP antibody response.

One such experiment is recorded in Table I. TDL from donors which had been primed with DNP-BGG were depleted of CRL and transferred together with
TABLE I

Effect of CRL Depletion of TDL on Adoptive Direct and Indirect Anti-DNP PFC Response

| TDL transferred | No. recipients | PFC/spleen (mean and range) |
|-----------------|----------------|----------------------------|
|                 |                | Direct                     | Indirect                   |
| 10⁷ Undepleted  | 5              | 1,670 (980-2,360)          | 58,400 (40,000-70,000)     |
| 10⁷ CRL depleted| 5              | 52 (10-100)                | 62,000 (50,000-73,000)     |

TDL collected from HO rats primed with DNPmo BGG. (HO × DA)F₁ recipients given TDL and DNPmo-BGG intravenously 6 h after 850 rad. CRL rosettes in TDL before depletion, 39%; and after depletion, 0.2% by rerosetting. Undepleted control TDL treated in parallel except that unsensitized SRBC were used instead of EAC in the rosetting mixture.

antigen into irradiated F₁ recipients. Direct and indirect PFC were assayed in the spleen 7 days later. Contrary to expectation the indirect PFC response was unaffected by CRL depletion, while the direct PFC response, although initially small, was markedly reduced by this procedure. The results of preincubation of the F₁, spleen cells with alloantisera and guinea pig C before PFC assay are shown in Table II and confirm the absence of a significant host contribution to either direct or indirect PFC responses. In two further experiments the direct PFC response after CRL depletion again fell to a few percent of the control level but the indirect PFC response only fell to 46 and 47% of the control response, respectively. Rerostetting the CRL-depleted TDL with EAC in these two experiments indicated that the depletion was more than 99.4% complete in both cases.

Abolition of the Adoptive Response by IgL Depletion. In view of the variability of the adoptive indirect PFC response after CRL depletion in the parent to F₁ system, additional evidence was sought for the absence of a host contribution in this semiallogeneic combination. TDL from primed donors were depleted of IgL after rosetting with rabbit Fab'₂-antirat Fab-coated SRBC. Table III shows that IgL depletion virtually eliminated the adoptive transfer of both the direct and indirect responses. As IgL-depleted TDL were shown subsequently to contain the helper cell population (Table V) these experiments established that irradiated F₁ recipients, receiving functionally active helper cells together with antigen, are incapable of producing significant numbers of PFC.

Effect of CRL Depletion in a Syngeneic Assay. The failure of CRL depletion of primed TDL to abolish the adoptive indirect PFC response does not accord quantitatively with the results of Parish and Hayward (2) employing a syngeneic system. Thus, in the semiallogeneic system the residual response, although variable, was never less than 46% of the control response from the undepleted population, while in the syngeneic system the response fell to 6% of the control value in a single recorded experiment.

This apparent discrepancy was resolved when two assays were compared directly. In the first of two experiments TDL from primed parental strain donors were depleted of CRL and transferred together with antigen into irradiated parental or F₁ recipients. PFC assays were carried out 7 days later. The second experiment was identical to the first except that undepleted TDL were transferred. In the semiallogeneic system the control TDL produced a response which
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TABLE II
Origin of Anti-DNP PFC in Spleens of Irradiated F1 Recipients after Transfer of Primed Parental Strain TDL, Spleens from Experiment in Table I

| Spleen suspensions pretreated with | Recipients of undepleted HO TDL | Recipients of CRL-depleted TDL |
|-----------------------------------|-------------------------------|-------------------------------|
| % Direct | % Indirect | % Direct | % Indirect |
| DAB/FCS on ice | 100* | 100* | 1 | 111 |
| Normal HO serum | 124 | 66 | 0 | 86 |
| Normal DA serum | 94 | 58 | 2 | 71 |
| HO anti DA serum | 107 | 59 | 1 | 79 |
| DA anti HO serum | 1 | 0.3 | 0 | 1.4 |

Pooled spleen suspensions from (HO × DA)F1, recipients shown in Table I incubated at 37°C with alloantisera and normal sera together with guinea pig complement (2). The cell suspensions were then assayed for PFC.

* Results expressed as percent of response given by control spleens (recipients of undepleted TDL) incubated on ice.

was only 36% of the control TDL in the syngeneic transfer. In contrast, the CRL-depleted TDL gave similar responses in both cases. Thus the semiallogeneic assay was relatively insensitive to the loss of those CRL which are indirect PFC precursors, a finding which has been regularly confirmed.

It could be argued that this comparison between the two assays is invalid since the contribution made by the host in the syngeneic transfer was unknown. To assess this contribution it was necessary to assay the indirect PFC response in irradiated rats given antigen and helper cells only. Table IV shows that removal of all IgL from primed TDL reduced the adoptive responses in a syngeneic transfer to less than 2% of the control while the corresponding response after CRL depletion was 19%. To establish that IgL depletion leaves helper function intact, recombination experiments were performed between primed IgL and IgL-depleted TDL from either primed or unprimed donors. The IgL were obtained from the SRBC pellet after separation of IgL from non-IgL on Isopaque/Ficoll. The experiment set out in Table V confirmed that IgL depletion leaves viable helper cells in the depleted population.

Dose-Response Relationships. The depletion experiments, in which the CRL in TDL were reduced from approximately 40 to <1% (from <0.1 to 0.3% in different experiments), appeared to confirm the existence of both CR+Ig+ and CR-Ig− indirect PFC precursors in rat TDL. However, if there was a gross excess of precursors over helpers in rat TDL the assay would be relatively insensitive to precursor cell depletion. This possibility seemed unlikely since an approximately 100-fold excess of precursor cells over helper cells would be required to account for the results and there would be difficulty in explaining the effectiveness of IgL depletion in eliminating the indirect PFC response. However an explanation of this kind was formally excluded by the experiment
TABLE III

Abolition of the Adoptive Anti-DNP PFC Response in Irradiated F₁ Recipients by Depleting Donor TDL of IgL; Absence of a Host Contribution to Response in a Parental → F₁ Transfer

| TDL transferred | No. recipients | PFC/spleen (mean and range) |
|-----------------|----------------|-----------------------------|
|                 |                | Direct  | Indirect  |
| 10⁷ Undepleted  | 5              | 3,200  | (1,440-3,880) |
| 10⁷ IgL depleted| 5              | 12     | (0-40)    |

TDL collected from HO rats primed with DNP₂₇-BGG. (HO × DA)F₁ recipients given TDL and DNP₂₇-BGG. PFC assays 8 days after cell transfer. IgL rosettes in TDL before depletion, 45%; and after depletion, <0.1% by rerosetting.

TABLE IV

Effect of CRL or IgL Depletion of TDL on Adoptive Indirect Anti-DNP PFC Response; Syngeneic Cell Transfer

| TDL transferred | No. recipients | Indirect PFC/spleen (mean and range) |
|-----------------|----------------|--------------------------------------|
| 10⁷ Undepleted  | 3              | 833,300 (636,800-944,000)             |
| 10⁷ IgL depleted| 3              | 16,300 (7,600-20,900)                 |
| 10⁷ CRL depleted| 4              | 154,400 (98,000-243,000)              |

TDL collected from HO rats primed with DNP₂₇-BGG. HO recipients given TDL and DNP₂₇-BGG. Rerosetting of TDL after depletion, <0.3% CRL; and <0.6% IgL.

Set out in Table VI. Varying numbers of hapten-carrier-primed TDL were combined with a fixed number of helper cells and transferred together with antigen into irradiated recipients. A significant antihapten indirect PFC response could be obtained only if more than 10⁷ TDL were transferred. It is clear that the assay is highly sensitive to the loss of indirect antibody-forming cell precursor (AFCP) since they are not present in large excess.

Relationship between CRL, IgL, and Large Lymphocytes in Rat TDL. Thoracic duct lymph in the rat contains a minor population of large, nonrecirculating lymphocytes which can be labeled with short courses of [³H]thymidine in vivo and in vitro (10, 16). A final set of experiments was performed to determine whether CR⁺Ig⁺ and CR⁻Ig⁺ AFCP in TDL were subpopulations of small lymphocytes or whether large, dividing lymphocytes contributed to either group.

The lymphocytes in TDL were labeled in vivo with [³H]thymidine (see Materials and Methods) and collected from the thoracic duct. CRL, FcRL, or IgL rosettes were prepared, separated on Isopaque/Ficoll, and assayed in a scintillation counter. It is apparent from Table VII that CRL and FcRL are to be found predominantly among the long-lived (lightly labeling), small lymphocyte population. Approximately 50% of the label was associated with the IgL.

Since the in vivo labeling experiments indicated that a substantial fraction of the large lymphocytes in rat TDL were CR⁻Ig⁺ it was necessary to determine whether these cells contributed to the indirect PFC response which was ob-
TABLE V
Detection of Helper Cells for the Adoptive Indirect Anti-DNP Response in TDL Depleted of IgL

| TDL transferred | No. recipients | Indirect PFC/spleen (mean and range) |
|-----------------|----------------|-------------------------------------|
| $5 \times 10^8$ B' + $6 \times 10^6$ T' | 3 | 25,230 (17,000-36,700) |
| $5 \times 10^8$ B' + $6 \times 10^6$ T° | 3 | 247 (200-290) |

TDL collected from HO rats that were either unprimed or primed with DNP$_{44}$-HSA/pertussis 11 wk earlier. (HO x DA)F~ recipients given TDL and DNP$_{44}$-HSA intravenously. B', primed B cells: IgL separated from primed TDL on Isopaque/Ficoll and recovered from pelleted rosettes by lysing SRBC with isotonic NH$_2$Cl/10% FCS. T', primed T cells: non-IgL from primed TDL. T°, unprimed T cells: non-IgL from unprimed TDL.

TABLE VI
Adoptive Indirect Anti-DNP PFC Response; B/T Dose-Response Relationship in Syngeneic Transfer

| TDL transferred | No. recipients | Indirect PFC/spleen (mean and range) |
|-----------------|----------------|-------------------------------------|
| $2 \times 10^6$ primed TDL | 2 | 1,525 (300-2,750) |
| $10^7$ primed TDL | 3 | 215,400 (172,900-249,500) |
| $10^7$ T | 4 | 488 (250-700) |
| $2 \times 10^6$ primed TDL + $10^7$ T | 4 | 1,300 (350-2,800) |
| $2 \times 10^6$ primed TDL + $10^7$ T | 4 | 31,550 (29,050-35,300) |

Primed TDL, from HO rats primed with DNP$_{44}$-BGG. T, IgL-depleted TDL from primed donors. Undepleted TDL run on Isopaque/Ficoll with normal SRBC before combining with T cells. HO recipients given cells and DNP$_{44}$-BGG.

served after the transfer of CRL-depleted TDL (Table I). TDL from primed donors were separated by sedimentation at 1 g into large and small cell fractions (11). The small cells, comprising 90% of all cells recovered and with 97% cell diameters less than 8 μm (as assessed by Coulter counter [Coulter Electronics Ltd., Dunstable, Bedfordshire, England] results) were subsequently depleted of CRL. The capacity of this doubly depleted population to transfer an adoptive PFC response was then assayed. As shown in Table VIII, neither direct nor indirect PFC responses were affected by removal of the large cells. In agreement with a previous finding (Table I), CRL depletion had a much more profound effect on the adoptive PFC response than on the indirect one.

Discussion
Lymphocytes forming rosettes with appropriately sensitized SRBC may be separated from nonrosetting cells by centrifugation on an Isopaque/Ficoll gradient (14). In the present study this technique has been applied to TDL from rats primed with DNP-BGG to determine the 19S and 7S precursor potential of cells bearing a receptor for activated C3 (CR° cells). The experiments have established that the precursors of 19S antibody-forming cells are present exclusively
TABLE VII

Radioactivity in Rosetting Cells after Labeling TDL In Vivo with [3H]Thymidine

| Rosettes | % Radioactivity in rosetting cells* |
|----------|----------------------------------|
|          | Exp. 1 | Exp. 2 |
| CRL      | 5.3    | 5.4    |
| FcRL     | 6.0    | ND     |
| IgL      | 45.6   | 79.6   |

* [(Activity in rosettes)/(Activity in rosetting + nonrosetting TDL)] \times 100. TDL from rats given [3H]thymidine (see Methods) were rosetted in two separate experiments. Rosettes separated from nonrosetting TDL on Isopaque/Ficoll. Autoradiographs prepared from smears of the TDL showed that 4% of the cells were labeled.

in the CR+ subpopulation. Those which give rise to 7S antibody-forming cells are present in both CR+ and CR- subpopulations, implying that the presence of a receptor for C3 is not essential for the induction of a secondary 7S response.

The conclusion that CR- cells act as 7S precursors follows from the observation that removal of CR+ cells from an immune population reduced, but never abolished, the adoptive 7S anti-DNP response. However, certainty about this interpretation required the exclusion of two other possibilities. Firstly, the existence of a radioresistant B cell in the rat (2) made it necessary to ensure that the 7S PFC response arose from the transferred CR- lymphocytes and not from the irradiated hosts. Secondly it was necessary to establish that the very small residual CR+ population that contaminated the CR- fraction after separation was inadequate to account for the magnitude of the response observed. In syngeneic transfers the donor origin of the PFC was inferred from the observation that depletion of Ig+ lymphocytes virtually abolished the adoptive 7S response, showing that the transfer of primed T cells alone did not confer secondary responsiveness. In contrast, the 7S response obtained from CR- populations were of similar magnitude in the two systems. These differences explain the relative insensitivity to CRL depletion of assays employing parent to F1 transfers. Inhibition of donor cell responses in hosts undergoing a graft vs. host reaction have been observed by others (17-19). As judged by the inability of CRL-depleted TDL to form a significant number of rosettes with EAC such depletions were over 99.5% complete. (It was established that exposure to Isopaque/Ficoll did not impair the capacity of undepleted TDL to rosette with EAC.) By supplementing a variable number of primed TDL with carrier-primed T cells and thereby obtaining a dose/response relationship it was shown that the very small number of CR+ cells remaining after CRL depletion was inadequate
TABLE VIII
Capacity of TDL Depleted of Large Lymphocytes and CRL to Transfer an Adoptive Anti-DNP PFC Response

| TDL transferred          | No. recipients | PFC response/spleen |
|--------------------------|----------------|---------------------|
|                          |                | Direct              | Indirect               |
| Normal*                  | Undepleted     | 5                   | 1,500 (290-2,040)     | 82,000 (40,380-186,000) |
|                          | CRL depleted†  | 5                   | 94 (40-200)           | 37,600 (23,000-143,800) |
| Small cell fraction§     | Undepleted     | 4                   | 1,200 (280-2,470)     | 78,000 (42,000-97,600)  |
|                          | CRL depleted†  | 5                   | 158 (70-260)          | 62,100 (20,000-75,500)  |

TDL collected from HO rats primed with DNP20-BGG. (HO x DA)F1 recipients given cells and DNP20-BGG intravenously.
* First 12-h collection of TDL.
† <0.5% CRL by rosetting.
§ Second 12-h collection of TDL from same donors depleted of large lymphocytes by sedimentation at 1 g.

to account for the residual 7S PFC response obtained in recipients of such TDL.

The CR-Ig+ cells in rat thoracic duct lymph comprise about 15% of the Ig+ population (reference 14 and unpublished data), that is, about 6% of all cells in the lymph. The observation that this small subpopulation contained virtually all the Ig+ blasts prompted the study of the effect of a combined CRL and large cell depletion on the capacity of primed TDL to transfer a 7S PFC response. The results of the double depletion indicated that the removal of CR-Ig+ blasts in rat TDL from a CRL-depleted population did not impair the capacity of the remaining cells to adoptively transfer a 7S PFC response. The Ig+ large lymphocytes in TDL are newly derived from lymphoid tissue draining into the thoracic duct; in normal animals the overwhelming contribution is from the intestinal lymphoid mass. The great majority of these Ig+ blasts carry surface IgA and migrate preferentially into the lamina propria of the gut (21). In the present experiments, the priming dose of antigen was given into the peritoneal cavity which does not engage lymphoid tissue draining into the abdominal portion of the thoracic duct. Thus, the lack of involvement of large lymphocytes in the adoptive 7S response in the present experiments was not unexpected.

The observation that approximately 50% of blast cells in rat TDL, defined by short-term in vivo labeling with [3H]thymidine are CR-FcR-Ig+ and that the majority, if not all, of the remaining blasts lack all three surface markers confirms a similar finding using in vitro labeling (14). However the claim that large lymphocytes shed their Fc and C3 receptors but retain their surface Ig during S phase was not supported by the present work. Basten et al. (21) reported that the cells which they classified as plasmablasts in the thoracic duct lymph of thymectomized, irradiated, marrow-restored mice did not possess Fc receptors and gut-homing Ig+ blasts in mouse mesenteric lymph nodes also lack C3 receptors (22).

If the contribution of the large lymphocytes is subtracted then the frequency of CR-Ig+ cells in the thoracic duct lymph of primed rats is very low (=1%). These cells were responsible for generating about 20% of the total 7S PFC
response given by undepleted TDL. In the following paper (23) the class of surface immunoglobulin on this small subpopulation is described in a study which aimed to determine the relation between the class of surface immunoglobulin on precursor cells and the class of immunoglobulin secreted by their differentiated progeny.

Studies on the relationship between CRL and 19S precursor cells encountered two difficulties in the present experiments. The magnitude of the adoptively transferred 19S response was always very much smaller than that of the 7S and in syngeneic transfers was dominated by a contribution from the irradiated host. The latter point was established by using an allotype-specific PFC assay in studies on cell transfer between congenic rats differing in their light-chain allotype (unpublished data). For this reason the effect of depleting TDL of CRL on the adoptive 19S response is reported only for semiallogeneic transfer. Such a depletion consistently reduced the 19S response to the level obtained by transferring IgL-depleted TDL. It is concluded that either 19S precursors are all within the CR⁺ population or CR⁻ precursors do not respond in a semiallogeneic transfer system. This problem is further examined elsewhere (23).

The function of the C3 receptor on lymphocytes has been extensively investigated (1, 4–8, 24–27). Although several hypotheses have been advanced (1, 4–8) no clearly defined role for this receptor has been established. The present work indicates that, since the secondary 7S response mediated by Ig⁺ CR⁻ precursor cells is T-cell dependent in the hapten-carrier system used, B-cell-T-cell cooperation is not invariably dependent on the presence of the C3 receptor on B cells. Further discussion on the possible role of the C3 receptor will be deferred until the results of the following paper have been presented.

Summary

The main conclusion from this study is that C3 receptors are not required for the generation from B cells of a thymus-dependent 7S antibody response. The requirement for C3 receptors on the precursors of antibody-forming cells was studied in an adoptive transfer system using thoracic duct lymphocytes (TDL) from primed rats as a source of precursors and irradiated recipients as hosts. 7S precursors were found in both the CR⁺ and the CR⁻ fractions of TDL and it was established that the response transferred by CR⁻ cells did not arise from either a radioresistant B cell in the host or from CR⁺ cells contaminating the CR⁻ population. Thus, the C3 receptor is not obligatory for B-cell-T-cell cooperation in the 7S response. The precursors of 19S antibody-forming cells were found only in the CR⁺ subpopulation. The CR⁻ Ig⁺ subpopulation was shown to contain all the B blasts in rat TDL and a very small number (approximately 1% of all TDL) of small lymphocytes. This latter population contained the CR⁻ 7S precursors and contributed approximately 20% of the total adoptive secondary 7S response transferred by CR⁺ and CR⁻ subpopulations combined. This observation suggests that the percentage of rat TDL committed to carry 7S memory is small, a conclusion which is confirmed and extended in the following paper.

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