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The Immune Reactivity of Lymph Node-Derived Spleen Colonies and Intercolonial Cells

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Summary. Using a method for cloning lymph node (LN) derived cells in the spleens of lethally irradiated mice, it was determined that the macroscopic colonies observed were haemopoietic whereas the intercolonial cells were primarily lymphoid. The immune response detected in isolated colonies was shown to be attributable to contamination of the clones by immunologically reactive interclonal cells. It was also demonstrated that pre-treatment of LN donor mice with PHA enhanced cloning efficiency but was not a necessary procedure for obtaining LN-derived colonies in the spleens of irradiated recipient mice.

INTRODUCTION

The selective theories of antibody production demand that the immune competent cell be predetermined to produce antibody of one or at most a very few specificities (Burnet, 1959; Lederberg, 1959). Several recently proposed theories of antibody production hypothesize such limited information in the genome of the immune competent cell (Brenner and Milstein, 1966; Cohen and Milstein, 1967; Edelman and Gally, 1967; Lennox and Cohn, 1967; Smithies, 1967; Whitehouse, 1967). Such hypotheses require the population of competent cells in any one organism to be heterogeneous in order to provide for the myriad of antibody specificities produced by a single individual. To maintain stability, a correlative condition for these theories requires that one competent cell produce a clone of progeny bearing identical information for antibody specificity (Burnet, 1959).

Experimentally induced clones of lymphoid cells would provide an ideal tool for determining whether each immune competent cell and its progeny are inherently capable of producing only one or a limited number of antibody specificities, or whether they can respond to any randomly chosen antigen. This latter would be predicted if the competent cell were endowed with a genome which coded for all possible antibody specificities (Szilard, 1960; Dreyer and Bennet, 1965), or one which was completely naive and required an external template in order to initiate antibody production (Haurowitz, 1965). Reports have been made of antibody producing 'foci' in the spleens of irradiated mice treated with lymph node or spleen cell inocula (Playfair, Papermaster and Cole, 1965; Kennedy, Siminovitch, Till and McCulloch, 1966). In this laboratory, Mekori, Chieco-Bianchi and Feldman (1965) and Feldman and Mekori (1966) have described a method for the *in vivo* culturing of macroscopic, antibody-producing 'lymphoid' clones in the spleens of lethally X-irradiated mice injected with lymph node cells from phytohaemagglutinin (PHA)-treated donor mice. It was our intention to analyse this experimental system in order to investigate the nature of the information for antibody specificity carried by a cloned

population of lymphoid cells by determining whether such 'lymphoid' clones are capable of producing antibody to randomly chosen antigens and whether an individual clone can produce antibodies to several different antigens.

MATERIALS AND METHODS

Animals

Two and a half- to 3-month-old mice of strains (C3H × C57BL)F_1, designated as C3H/BL; (BALB/CC × DBA/2)F_1, designated as CD_2; (BALB/CC × C57BL) F_1, designated as BALB-BL; and C57BL, of both sexes were used as recipients of cell inocula for clone formation. Two- to 2½-month-old donors of lymph node (LN) cells and 6-week-old donors of bone marrow (BM) cells were of the same sex and strains as the recipients.

X-irradiation

Mice in leucite containers were irradiated with a General Electric Maximar III X-ray machine (250 kV, 15 mA), at a target distance of 50 cm (dose rate 65 r/min) with 0·5 mm Cu and 1 mm Al filters. CD_2 mice were exposed to 900 r total body irradiation; all the other strains used, to 850 r.

Cloning procedure

In accordance with the procedure for the production of 'lymphoid' clones (Mekori *et al.*, 1965; Feldman and Mekori, 1966), recipient mice were lethally irradiated and, within 3 hours of irradiation, inoculated intravenously (i.v.) with 50×10^6 LN cells from the cervical, axillary, brachial, inguinal and mesenteric LNs of isologous donors. In certain of the experiments to be reported, the donors of LN cells received an i.v. injection of 0·3 ml PHA-M (Difco) 3 days prior to the removal of their LNs. Suspensions of cells were obtained by passing the LNs through a stainless steel mesh of pore size 0·6 × 0·6 mm into cold Tyrode's solution. The suspensions thus obtained were passed once through a 27-gauge needle to ensure complete dispersal of the cells. All LN cell suspensions were washed once in Tyrode's solution to remove fat globules which would otherwise have caused fatal embolisms in recipient mice.

Clones of bone marrow origin were produced by i.v. inoculation of lethally irradiated recipient mice with 10^4 BM cells in Tyrode's solution. These cells were obtained by flushing the tibia and femur of donor mice with cold Tyrode's solution.

After 10 days of growth in the spleens of lethally irradiated recipient mice, clones were dissected out under ×8 magnification (Nikon SMZ dissecting stereomicroscope) with ophthalmic scissors and fine forceps. In experiments with intercolonial tissue, care was taken to isolate such tissue from areas of the spleen in which no macroscopic colonies were observed.

Antigens

Sheep, camel and rabbit RBC were maintained in Alsever's solution (Kabat and Mayer, 1961) at 4°. Before use, the RBC were washed three times in saline (0·9 per cent NaCl). Mice were immunized by a single intraperitoneal (i.p.) injection of 4×10^8 RBC. *Shigella paradys.*, prepared according to the method of Harris, Harris and Farber (1954), was injected i.p. in a dose of 0·2 ml of a 0·1 per cent suspension in saline.

Immunization schedule

In experiments using erythrocyte antigens, mice were immunized on day 5 following irradiation and LN cell inoculation, and their response assayed by the haemolysin plaque technique (Jerne, Norden and Henry, 1963) on day 10 post-inoculation. Control groups included unirradiated normal mice and irradiated mice which were not given LN cell inocula. Both of these groups were immunized and assayed according to the schedule described above. In experiments with *Shigella* antigen, lethally irradiated LN inoculated mice (primary recipients) were immunized on day 6 post-irradiation. Their spleens were removed and cells of colonial or intercolonial origin were transferred to lethally irradiated secondary recipients on day 10, as described by Feldman and Mekori (1966). Certain of the secondary recipients were given a booster immunization on day 3 after secondary transfer and 5 days later all mice were bled from the supra-orbital sinus. Controls for this series of experiments included immunized unirradiated mice and irradiated mice recipients of immunized normal spleen cells, BM cells, or spleen cells from lethally irradiated *Shigella*-treated mice which had not been subjected to an inoculation of LN cells. The immunization and bleeding schedules of these control groups of mice were the same as that of the experimental groups. The individual serum samples thus obtained were assayed for anti-*Shigella* agglutinins as described below.

Assays

The haemolytic plaque assay of Jerne *et al.* (1963) was performed on plastic Petri dishes (60 × 15 mm, Falcon) with Difco bacto-agar in Hanks's solution without bicarbonate, plus DEAE-Dextran (Pharmacia). Samples of colonies, or other splenic tissue were suspended in Hanks's solution by dispersing the cells with a hypodermic syringe and a 27-gauge needle. The plaques were developed by flooding the plates with 2 ml of a 1 : 25 dilution of guinea-pig complement (Texas Biological Laboratories, Inc., Fort Worth, Texas) in Kolmer's saline.

The assay for circulating anti-*Shigella* agglutinins was carried out according to the method described by Feldman and Mekori (1966).

The Simonsen graft-*versus*-host (GVH) assay was performed by injecting 0·1 ml of the suspension of cells to be tested for their immune competence i.p. into newborn F_1 hybrid mice. Cell suspensions were obtained as described for the haemolysin plaque technique with the substitution of Tyrode's for Hanks's solution. On day 11, the recipients were killed and their spleen indices computed by the method of Simonsen, Engelbreth-Holm, Jensen and Poulsen (1958).

Cell counts

RBC were suspended in saline (0·9 per cent NaCl) for counting in a haemocytometer. Total cell counts of nucleated cell samples were performed in Turk's solution (0·5 ml 1 per cent Gentian Violet in distilled water; 1 ml acetic acid, 100 ml distilled water). Viable cell counts were performed by the Trypan Blue exclusion test using 0·1 g Trypan Blue in 200 ml Tyrode's solution.

Histology

Spleen samples, fixed in Bouin's solution, were sectioned in paraffin blocks and stained with haematoxylin and eosin.

RESULTS

An initial series of experiments performed with the 'lymphoid clones' was designed to determine the capacity of each individual clone to evince an immune response to randomly chosen antigens. Since our interest was in quantifying the response of individual clones by determining the number of cells per colony capable of a response, we chose to use erythrocytes of various species as antigen in the haemolysin plaque technique assay. As can be seen from the results recorded in Table 1, almost every immunized clone tested, regardless of strain of mouse or antigen used, evinced an immune response. The background response recorded by the control unimmunized clones probably represents natural antibody plaques which are also observed in normal unimmunized mice (Friedman, 1964).

As control groups in some of the experiments reported in Table 1, lethally irradiated recipient mice were injected with suspensions of LN cells from donors which had not been treated by PHA. We found that the spleens of such mice bore colonies, albeit in lower frequency than after PHA treatment (Table 2). Macroscopically these colonies could not be differentiated from colonies formed after PHA treatment of donor cells.

To differentiate between the colonies derived from PHA treated or untreated LN cells, histological preparations were made of the two types of colonized spleens as well as spleens of all control groups. The spleens of X-ray control mice which had not received the LN inoculum were severely depopulated. Such spleens, whether from mice which had been immunized or not, contained greatly atrophied, minute foci of lymphoid cells surrounding

TABLE 1

RESPONSE OF 'LYMPHOID' CLONES FROM MICE OF VARIOUS STRAINS AND BOTH SEXES STIMULATED WITH ERYTHROCYTE ANTIGENS

Mouse strain	RBC antigen	Immune clones*		Control clones†	
		Responders/ total tested	Average PFC/ 10^6 cells	Responders/ total tested	Average PFC/ 10^6 cells
CD_2, female	Camel	7/14	182	1/10	1·8
CD_2, male	Rabbit	10/10	38	3/7	1·5
CD_2, male	Sheep	9/9	103	5/6	2·2
C3H/BL, female	Camel	6/6	39	0/6	0
BALB-BL, male	Sheep	22/22	206	11/21	1·8

* Colonies isolated from the spleens of lethally irradiated mice which had received an i.p. inoculum of RBC, 5 days after transfer of LN cells from PHA treated donors.

† Colonies isolated from spleens of lethally irradiated mice recipients of an inoculum of LN cells from PHA treated onors, but not exposed to the RBC antigen.

TABLE 2

THE EFFECT OF PHA ON THE CLONING EFFICIENCY OF LYMPH NODE CELLS IN THE SPLEENS OF LETHALLY IRRADIATED RECIPIENT MICE

Mouse strain	No. of recipient mice	Total colonies counted	Colonies/10^7 viable LN cells injected
CD_2, male Treated with PHA	80	430	2·7
Not treated with PHA	71	279	1·5
C3H/BL, female Treated with PHA	160	278	0·52
Not treated with PHA	80	40	0·18

CD_2 mice were irradiated with a dose of 900 r and C3H/BL mice with 850 r; both received an i.v. inoculum of 50×10^6 LN cells of which from 30×10^6 to 37×10^6 were viable as determined by the Trypan Blue exclusion test.

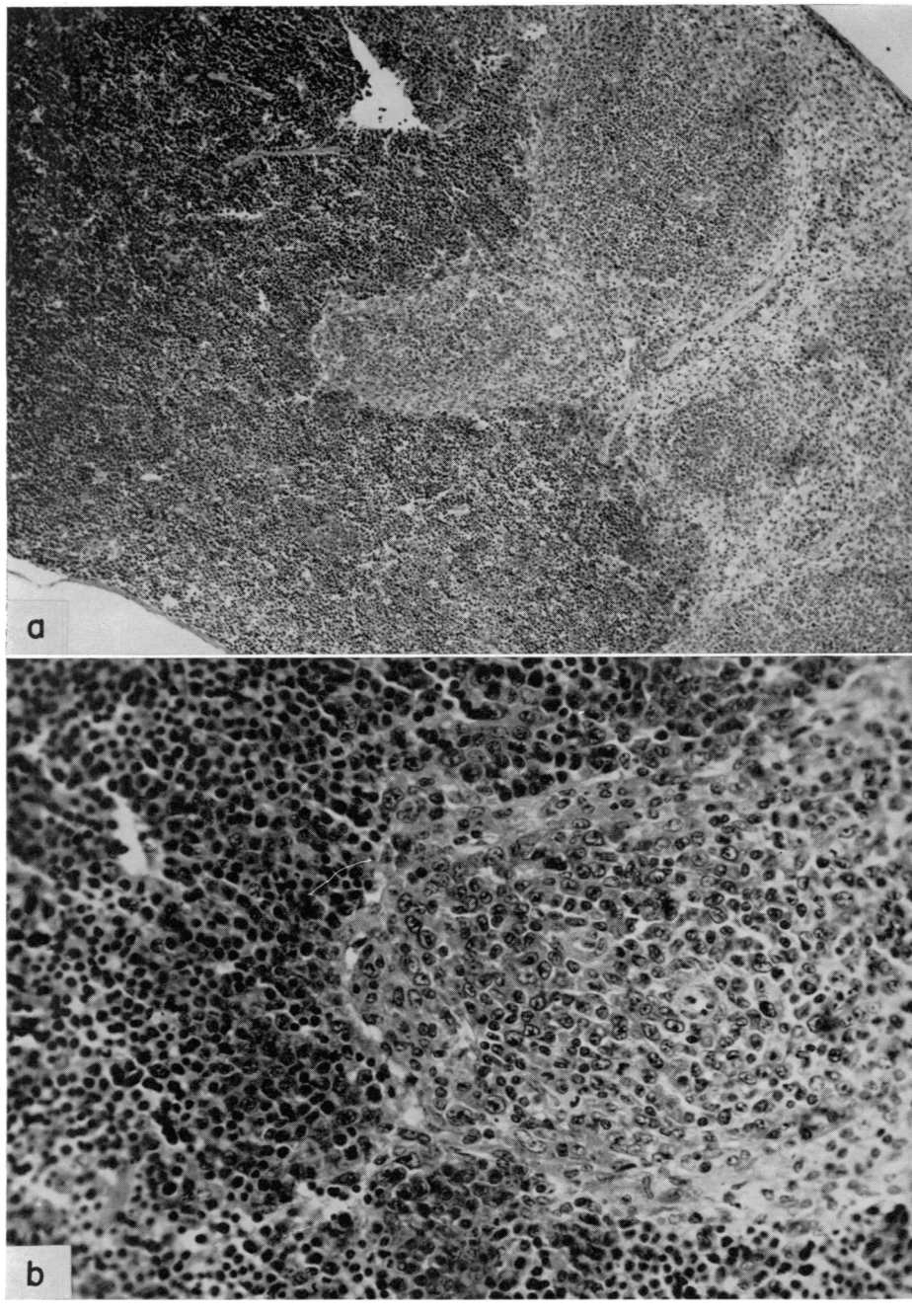

FIG. 1. Erythroid clone surrounding population of lymphoid cells. Note dark pyknotic nuclei of the erythroid cells. (a) × 350. (b) × 1400.

TABLE 3

A COMPARISON OF THE ANTI-SRBC RESPONSE OF COLONIAL AND INTERCOLONIAL TISSUE, WITH AND WITHOUT PRETREATMENT OF LN DONORS WITH PHA

Mouse strain	Tissue origin	PHA treatment of donor mice	No. of samples tested	Total No. of cells plated ($\times 10^6$)	Total No. of plaques counted	Average No. of PFC/10^6 cells
CD_2, male	Colony	+	37	23·8	793	33
	Intercolonial	+	34	27·7	1,789	65
	Colony	−	33	17·5	679	39
	Intercolonial	−	27	11	1,348	123
C3H/BL, male	Colony	−	7	2·2	143	64
	Intercolonial	−	7	0·44	236	539
C3H/BL, female	Colony	−	9	3·7	375	101
	Intercolonial	−	9	4·1	1,201	291

All lethally irradiated LN cell recipient mice were immunized with 4×10^8 SRBC, 5 days after irradiation and LN cell transfer. Colonial and intercolonial cells were assayed for PFC on day 5 after immunization.

vascular tissue. Histological examination of spleens bearing colonies derived from PHA treated or untreated LN cells, left us unable to differentiate between the two types of spleen. The colonies, macroscopically visible on the surface of the spleen, were observed histologically to be populated by cells predominantly of the erythroid or granuloid cell lines, with occasional megakaryocytes. Another observation was the dense lymphoid repopulation of the intercolonial tissue of these spleens; lymphoid cells in all stages of development were prevalent, and plasma cells were often observed. Upon further study of these preparations, we observed many areas where the haemopoietic cells of the colonies had proliferated in such a manner as to surround, or partially surround, large populations of lymphoid tissue (Fig. 1). This histological picture aroused great suspicions about the validity of the original assumptions concerning the nature of the colonies we had been isolating for study. The histological preparations forced us to conclude that it was impossible to isolate colonies clean of the dense population of background lymphoid cells. They also suggested that the immune response we were detecting in the so-called 'lymphoid' clones was not elicited by the major cell population of the colony, which was haemopoietic, but rather by a contaminant of lymphoid cells from the background, intercolonial tissue. To test this hypothesis, we performed a series of experiments in which we isolated colonies and intercolonial tissue from the spleens of mice which had been inoculated with 50×10^6 LN cells from two donor groups, one treated with PHA and the other not. Table 3 represents the cumulative results of seven separate experiments. It was repeatedly demonstrated that the intercolonial tissue responded with more plaque-forming cells (PFC) per million cells than did the colonial tissue. In individual experiments, this intercolonial response was detected to be from two to nine times higher than that of the colonies. These results agreed well with our interpretation of the histology of the colonized spleens.

The above results provided us with a slight dilemma. In the experiments performed previously in this laboratory (Feldman and Mekori, 1966), the intercolonial tissue was found incompetent to respond immunologically. The 'interclonal' cells which were tested in those studies were taken solely from the spleens of LN cell treated, irradiated mice in which colonies had not developed. Using SRBC and the haemolytic plaque assay, we found that 'interclonal' material from such spleens, which had not developed colonies, behaved in the same manner as true intercolonial tissue. This, therefore, could not be the

TABLE 4

RESPONSE OF COLONIES AND INTERCOLONIAL CELLS TO *Shigella* ANTIGEN: LACK OF RESPONSE OF INTERCOLONIAL CELLS

C3H/BL, female mice X-rayed with 850 r and inoculated with:	No. of cells transferred to secondary recipient ($\times 10^6$)	No. of samples tested	Per cent responders	Average anti-*Shigella* agglutinin titre ($1/\log_2$)
Immune colony and BM	1 colony	15	13	0·6
Immune colony and BM and *Shigella*	1 colony	19	37	1·6
Immune intercolonial cells and BM	5·9	7	0	0
Immune intercolonial cells and BM and *Shigella*	5·9	26	4	0·08
Immune normal spleen* and *Shigella*	7	19	100	8·7
Immunized irradiated spleen and *Shigella*	2·8	8	0	0
10^6 BM and *Shigella*	0	18	0	0
Shigella	0	10	0	0
Immunized normal control† (not transferred)	–	22	100	9·5

BM = 10^6 normal bone marrow cells.

* Primary immunization given at same time as to primary hosts.

† Immunized at same time as secondary hosts.

TABLE 5

RESPONSE OF COLONIES AND INTERCOLONIAL CELLS TO *Shigella* ANTIGEN: POSITIVE RESPONSE OF INTERCOLONIAL CELLS

X-rayed mice inoculated with:	No. of cells transferred to secondary recipient ($\times 10^6$)	No. of samples tested	Per cent responders	Average anti-*Shigella* agglutinin titre ($1/\log_2$)
Immune colony and BM	1 colony	37	0	0
Immune colony and BM and *Shigella*	1 colony	34	38	1
Immune intercolonial cells and BM	7·2	45	22	0·4
Immune intercolonial cells and BM and *Shigella*	7·5	34	88	5·3
Immune normal spleen*	7·1	25	36	0·9
Immune normal spleen* and *Shigella*	8·3	19	95	12·4
Immunized irradiated spleen and *Shigella*	6	25	28	0·7
10^6 BM and *Shigella*	0	30	0	0
Normal controls:				
Primary response*	—	21	100	11
Secondary response†	—	28	100	14·2

Results of experiments performed on C3H/BL females and CD_2 males. The data from these two strains were pooled since they did not differ significantly. The C3H/BL mice received 850 r total body irradiation and the CD_2, 900 r.

BM = 10^6 normal bone marrow cells from isologous donors.

* Primary immunization given at same time as to primary hosts.

† Primary immunization given at same time as to primary hosts and booster given at time of immunization of secondary hosts.

cause of the discrepancy in our results. We then questioned whether differences in antigen or assay might have any bearing on our results, and so we proceeded to repeat the experiments of Feldman and Mekori (1966), using their antigens and assays.

In testing the response of colonial and intercolonial cells to *Shigella paradys.*, our results were variable. In two out of six experiments performed, the intercolonial tissue demonstrated no immune response, whereas the colonial tissue did elicit a weak agglutinin

reaction (Table 4). It should be noted that in these experiments the average agglutinin titres produced by the colonial material as well as the numbers of colonies responding were very much less than those reported previously (Feldman and Mekori, 1966). In the remaining four out of six experiments (Table 5), positive agglutinin titres were obtained from intercolonial cells, especially if the secondary host received a booster immunization of *Shigella*. This dependence upon immunization in the secondary host appears to be characteristic of the assay, since spleen cells from immunized unirradiated mice, transferred to irradiated hosts, did not produce significant titres of circulating antibody unless they were provided with a secondary immunization in the host animal (see Table 5). Once again in this series of experiments we found the response of the colonized cells much lower than that reported previously (Feldman and Mekori, 1966). The results of this series of experiments supported our contention that the intercolonial tissue in spleens of irradiated mice, treated with 50×10^6 LN cells, is competent to elicit an immune response.

The response of intercolonial cells in the Simonsen GVH assay gave further confirmation to our hypothesis concerning the immune competence of intercolonial tissue (Table 6). Eight litters of seven to nine newborn C3H/BL mice were inoculated i.p., within 24 hours of birth, with 5×10^6 cells of the following origins: colonies, intercolonial cells, normal C57BL spleen, and irradiated C57BL spleen. In each litter, one or two mice were left untreated to serve as normal reference mice in computing the spleen index. To provide 5×10^6 cloned cells, several colonies were pooled. The colonies were produced by inoculating lethally irradiated (850 r) C57BL female mice with 36×10^6 Trypan Blue excluding isologous cells. In two of the four experiments performed, donor mice were treated with PHA and in the other two, they were untreated. The indices recorded in the Simonsen assay did not differ significantly in the two groups; therefore, the results were pooled in Table 6.

The results of the above series of experiments confirmed that intercolonial cells are competent to elicit an immune response. None the less, we were still faced with the question of the origin of the immune competent cells within isolated colonies. Were these cells progeny of the cloned cell, or were they simply a contaminant from the surrounding lymphoid tissue? The histology of the colonized spleens seemed to suggest the latter. The following reconstruction experiment was performed to determine whether one can obtain a positive immune reaction from an immunological incompetent haemopoietic clone which is situated in a background of potentially contaminating, immunologically active cells. We have confirmed experimentally the report (Mekori and Feldman, 1965) that intrasplenic clones of haemopoietic cells produced by an i.v. injection of BM cells into a lethally irradiated host are immunologically incompetent, as are spontaneous post-irradiation clones. We, therefore, designed experiments to test whether such clones can be

TABLE 6

THE COMPETENCE OF 'LYMPHOID' CLONES AND INTERCOLONIAL TISSUE TO CAUSE SPLENOMEGALY IN THE SIMONSEN GVH ASSAY

Newborn C3H/BL mice injected with:	No. of mice	Per cent with splenomegaly	Average spleen index	Range of indices
Normal control (untreated)	14	0	1·00	0·89–1·11
Intercolonial cells	15	93	2·38	1·26–3·67
Clonal cells	12	75	1·91	1·12–2·92
C57BL spleen cells	9	100	2·42	1·79–3·18
C3H/BL spleen cells	8	0	1·08	0·88–1·26
Irradiated C57BL spleen cells	4	0	1·23	1·07–1·40

Indices above 1·50 were taken to represent splenomegaly and, therefore, the presence of a GVH reaction.

TABLE 7

THE NUMBER OF CLONES PRODUCED BY VARIOUS CELL INOCULA IN LETHALLY IRRADIATED C3H/BL FEMALE MICE

Inoculum	No. of mice	No. of viable BM cells injected ($\times 10^4$)*	No. of viable LN cells injected ($\times 10^6$)*	No. of clones observed	No. of clones expected from:		
					BM	LN	Spontaneous†
50×10^6 LN	41	—	146·5	28	—	16·9	11·1
20×10^6 LN	40	—	70·6	12	—	1·2	10·8
10^4 BM	48	48	—	125	112	—	13
10^4 BM and 20×10^6 LN	42	42	74·8	100	98‡	1·3§	11·5
X-rayed control	22	—	—	6	—	—	6

* Counted as Trypan Blue negative
† Based on results from X-ray controls.
‡ Based on results from BM inoculum.
§ Based on results of 20×10^6 LN inoculum.

TABLE 8

DEMONSTRATION OF IMMUNE REACTIVITY INDUCED IN IMMUNOLOGICALLY INCOMPETENT CLONES BY CONTAMINATION WITH LYMPHOID CELLS OF THE INTERCOLONIAL BACKGROUND

Group	No. of samples plated	Total No. of cells plated ($\times 10^6$)	Total No. of plaques counted	Average No. of PFC/10^6 cells
X-ray, 5×10^7 LN, SRBC				
Clone	17	13·5	1,157	86
Interclone	27	20·9	13,843	663
X-ray, 2×10^7 LN, SRBC				
Clone	12	10·6	281	26
Interclone	39	40·8	4,468	110
X-ray, 10^4 BM, SRBC				
Clone	37	37·9	83	2
Interclone	35	33·6	207	6
X-ray, 10^4 BM, 2×10^7 LN, SRBC				
Clone	40	31·0	2,754	91
Interclone	34	31·1	6,337	202
X-ray, SRBC				
Spleen cells	15	25·2	46	2
Normal control, SRBC	8	8·8	2,832	322

C3H/BL female mice, exposed to 850 r total body irradiation, were recipients of cell inocula.

infiltrated by, or overgrow and include, lymphoid cells provided by an inoculum of LN cells, which, in itself, is too small to produce colonies. The following experimental protocol was used: Lethally irradiated (850 r) C3H/BL female mice were inoculated within 3 hours post-irradiation with either 10^4 BM cells, 50×10^6 LN cells, 20×10^6 LN cells, or 10^4 BM cells plus 20×10^6 LN cells. Five days later, all groups of mice were immunized with 4×10^8 SRBC i.p. Five days later still, the recipient mice were killed, their spleens removed, clones counted, and colonial and intercolonial cells plated in the Jerne plaque assay. From Table 7 it can be seen that the number of clones detected in the mixed BM and LN group is a close approximation of the sum of the corresponding individual groups. Almost all of these colonies can be attributed to either BM or spontaneous post-irradiation origin, both of which, by themselves, are known to be immunologically incompetent (Mekori and Feldman, 1965). Table 8 demonstrates the excellent immune response elicited by the

colonies of the mixed BM–LN inoculum, indicating that erythroid, granuloid and megakaryocyte clones situated in a milieu of immune competent lymphoid cells can grow and encompass some of these cells in such a way that it is impossible to dissect clones free of lymphoid contamination even with the aid of a dissecting microscope. It should be noted here that histological sections of the mixed BM–LN spleens were undistinguishable from those obtained with 50×10^6 LN cells alone.

DISCUSSION

In agreement with the report of Micklem (1966), we have found that pre-treatment of donor mice with PHA is not a necessary prerequisite for the formation of macroscopic spleen colonies in lethally irradiated recipients of LN cells. However, such treatment does enhance the cloning efficiency of the LN inoculum. It has been suggested that this type of enhanced colonization may be a reflection of an increased number of circulating haemopoietic stem cells (Micklem, 1966).

Histological studies of the spleens of lethally irradiated mice colonized by inocula of LN cells from PHA-treated or untreated donors led us to conclude that the cells situated between the macroscopically visible colonies were lymphoid and that the colonies were erythroid, granuloid and megakaryocyte in nature with some lymphoid elements in them. Further studies, utilizing the Jerne plaque assay, as well as the Simonsen GVH assay and bacterial agglutination, determined the high level of immune reactivity of the intercolonial tissue as well as the ability of the cell population isolated as clones to react immunologically.

A reconstruction experiment was performed to test whether immunologically incompetent colonies of BM origin or spontaneous nature, grown in splenic tissue simultaneously with lymphoid cells, can be contaminated by the lymphoid cells and, therefore, elicit a detectable haemolysin response in the Jerne plaque assay. This was found to be the case.

We, therefore, conclude that the LN-derived colonies originally denoted as 'lymphoid' are not lymphoid but rather haemopoietic. In all probability, the lymphoid elements dissected out with the clone are not part of the clone itself, but rather a contaminant from the intercolonial background of lymphoid cells. In the light of these conclusions we must abandon the hope of using this system of LN-derived colonies to study the quality and quantity of information for antibody specificity carried by the immune competent cell and its cloned progeny.

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