Clin Exp Immunol 1997; **109**:356–361

Human peritoneal B-1 cells and the influence of continuous ambulatory peritoneal dialysis on peritoneal and peripheral blood mononuclear cell (PBMC) composition and immunoglobulin levels

H. H. DONZE*, C. LUE*†, B. A. JULIAN†, W. H. KUTTEH‡, A. KANTELE* & J. MESTECKY*† *Departments of *Microbiology and †Medicine, University of Alabama at Birmingham, Birmingham, AL, and ‡Department of Obstetrics and Gynaecology at University of Tennessee Health Sciences Centre, Memphis, TN, USA*

(Accepted for publication 30 April 1997)

SUMMARY

In mice, peritoneal B cells are composed of a unique B-1 cell population which can repopulate the intestinal lamina propria with IgA-producing cells, as well as contribute to the majority of serum IgM. In this study, peritoneal lymphocytes from patients starting continuous ambulatory peritoneal dialysis (CAPD) and from women undergoing bilateral tubal ligation (BTL) were analysed for the presence of a B-1 cell population as well as the expression of potential homing receptors. Up to 63% of the peritoneal B cells express surface antigen CD5, and most peritoneal lymphocytes express the mucosal homing receptors, $\alpha 4\beta 7$ and $\alpha E\beta 7$. When analysing serial samples collected from patients from the beginning of dialysis to 1 year, no marked changes were observed in serum or salivary immunoglobulin levels, although the peritoneal lymphocyte population was reduced by 50%. These data suggest that the phenotype of human peritoneal B-1 cells is similar to that of mice, but the contributions to the immune system may differ.

Keywords B-1 cells peritoneal lymphocytes continuous ambulatory peritoneal dialysis homing receptors mucosal immunology

INTRODUCTION

Lymphoid cells of the mouse peritoneal cavity contribute to both mucosal and systemic compartments of the immune system [1]. The mouse peritoneal cavity contains a distinct lineage of B lymphocytes known as B-1 cells, which differ from conventional B-2 cells by their surface markers and origin [1–4]. B-1a cells express the surface marker CD5 and constitute a significant proportion of the peritoneal B cells, but are rarely found in peripheral blood or bone marrow [5–8]. Peritoneal B-1 cells can give rise to IgA-producing plasma cells in the mouse intestine [3,9–12], and depletion of this cell population by chronic peritoneopheresis or treatment with anti-IL-10 antibody significantly decreases serum IgM levels [13,14].

In humans, B-1 cells are found in low numbers in both the peripheral blood and bone marrow [15,16]. During fetal development the omentum serves as a reservoir for B-1 cells [17], but the percentage of B-1 cells is diminished in adulthood [18]. The role that B-1 cells play in the human immune system is not fully understood [8,18,19]. In a previous study, we identified B-1 cells in the peritoneal fluid of patients who had been on continuous ambulatory peritoneal dialysis (CAPD) for 2 years [20,21].

In this study, we characterized the peritoneal B-1 population and their homing potential based on surface marker expression as determined with flow cytometry from patients from the initiation of CAPD, as well as from healthy women undergoing bilateral tubal ligation (BTL). Because peritoneopheresis of the mouse peritoneal cavity is analogous to CAPD [13,22], we investigated the influence of long-term continuous dialysis on the composition of peritoneal and peripheral blood mononuclear cells (PBMC), as well as its influence on serum and salivary immunoglobulin levels.

Correspondence: H. Hale Donze, University of Alabama at Birmingham, Department of Microbiology, 746 Bevill Biomedical Research Building, 845 19th St South, Birmingham, AL 35294, USA.

PATIENTS AND METHODS

Study group

The study population consisted of two groups, 11 CAPD patients (four black females, four white females, and three black males) starting CAPD due to end-stage renal failure and 16 healthy women (eight black and eight white) undergoing BTL for reasons other than endometriosis. The median age of the groups was 51

years (range 25–75 years) and 27 years (range 21–38 years), respectively. Four of the CAPD patients were followed for a period of 1 year. The patients were seronegative for HIV antibodies and hepatitis B antigen, and the CAPD patients did not experience any episodes (e.g. peritonitis) during the duration of the study. CAPD patient no. 1 was seronegative for HIV at 52 weeks; however, this patient seroconverted within 6 months after the last sample. Approval for this study was given through the Institutional Review Board, and informed consent was obtained from all participants.

Sample collection

The peritoneal dialysate was collected from the CAPD patients by gravity drainage [22] and used as the source of the peritoneal cells. The total time in which the dialysate was exposed to the peritoneal cavity ranged from 4 to 8 h. For analysis of PBMC, peripheral blood was drawn from the CAPD patients in heparinized vacutainers; one non-heparinized tube was taken to obtain serum. Whole saliva (unstimulated) was collected into polypropylene tubes. In the three patients that were followed from the initial CAPD through 1 year, peritoneal dialysate, saliva and serum were serially collected at 0, 2, 4, 6, 12, 26 and 52 weeks, and from an additional patient, peritoneal fluid and saliva were collected. During the laproscopic BTL procedure, the cul-de-sac of healthy women was irrigated with ≈ 10 ml of saline and aspirated into 15-ml conical tubes containing heparin. Peripheral blood was collected as described above.

Preparation of samples

Blood drawn in non-heparinized tubes was allowed to clot and the serum was collected and frozen at −20°C. Saliva was centrifuged and frozen at −20°C. Peritoneal cells from BTL and CAPD patients were isolated by centrifugation as described previously [20]. PBMC were isolated over a Ficoll–Hypaque gradient. Cells were resuspended and viability was determined by trypan blue exclusion. Differential counts were performed after staining with Wright's reagent.

Analysis of lymphocyte phenotype

Two-colour flow cytometry was used to determine the phenotypes of the peritoneal lymphocytes and PBMC. Cells were stained with MoAbs against human CD3 (pan-T cell marker), CD19 (pan-B cell marker), and CD5 (pan-T and B-1 cell markers). Three-colour flow cytometry was used to detect homing receptor expression on the peritoneal cell population and PBMC from the same patient. Cells were stained using MoAbs against the gut homing receptor $\alpha 4\beta 7$ (ACT-1 [23]; kind gift from LeukoSite Inc., Cambridge, MA), mucosal-associated integrin $\alpha E\beta 7$ (CD103, HML-1; Immuntech, Marseille, France), or the peripheral lymph node (PLN) homing receptor L-selectin (CD62L, Leu-8), and co-stained with antibodies against CD5 and CD19. Isotype-matched control antibodies were used to demonstrate any non-specific staining. All antibodies were purchased from Becton Dickinson (San Jose, CA) unless stated otherwise.

Analysis of immunoglobulin production

Cells were stained for cytoplasmic IgA, IgG and IgM using a three-colour immunofluorescence technique described previously [24]. Total IgM, IgA, and IgG levels in the peritoneal effluent, saliva and serum were measured by ELISA. Briefly, 96-well polyvinyl microtitre plates (Dynatech Labs, Chantilly, VA) were coated with the $F(ab')_2$ fragments of goat anti-human IgG 2·5 μg/ml, IgA 2·5 μg/ml, or IgM 1·0 μg/ml (Jackson ImmunoResearch, West Grove, PA). Biotinylated goat anti-human IgA, IgG, and IgM (Tago Immunologicals, Burlingame, CA) were used as secondary antibodies. A serum pool (Moni-Trol E; Baxter, McGraw Park, IL) with known concentrations of isotypes served as a standard for total IgA, IgG, and IgM levels. Standard curves were constructed using a computer program based on the four-parameter logistic model (Delta Soft, BioMetallics Inc., Princeton, NJ). Serum immunoglobulin levels which are reported in Table 3 were determined by nephelometry [25].

RESULTS

Differential cell counts and phenotypic analysis

Most peritoneal cells were classified as monocytes in both patient groups; the second largest population consisted of lymphocytes. The polymorphonuclear cell (PMN) count was low in both the BTL and CAPD peritoneal populations, indicating no local acute inflammation (Table 1).

The phenotypes of the lymphocyte populations isolated from both the initial peritoneal effluent of the CAPD group and peritoneal wash of the BTL patients were assessed by flow cytometry analysis. The percentages of T and B cells, as determined by anti-CD3 and anti-CD19 reagents (Table 1), revealed that the T cells

Table 1. Phenotype of peritoneal lymphocytes*

Patient group	No. of cells ($\times 10^6$)	Monocytes† (%)	Lymphocytes† (%)	PMN† (%)	Other†§ (%)	$CD3^+$ T cells‡ (%)	$CD19^+$ B cells‡ (%)
CAPD ($n = 11$)	6·4 (0·14–380)	49 (23–86)	26 (4–49)	7 (1–61)¶	6 (0–30)	40 (8–61)	2 (0·5–10)
BTL ($n = 11$)	3·3 (0·16–82)	65 (36–100)	28 (0–57)	1 (0–28)	0 (0–4)	56 (10–79)	8 (1–16)

* Results are expressed as median values (range).

† The phenotype was differentiated after staining with Wright's reagent.

‡ The phenotype was determined by FACS staining.

§ Other includes peritoneal mesothelial cells and non-classified cells.

¶ The PMN count in 10 patients was below 18%. In one patient, 61% of the cells were PMN; however, this patient did not present any clinical signs of peritonitis and the peritoneal fluid cultures were negative.

CAPD, Continuous ambulatory peritoneal dialysis; BTL, bilateral tubal ligation.

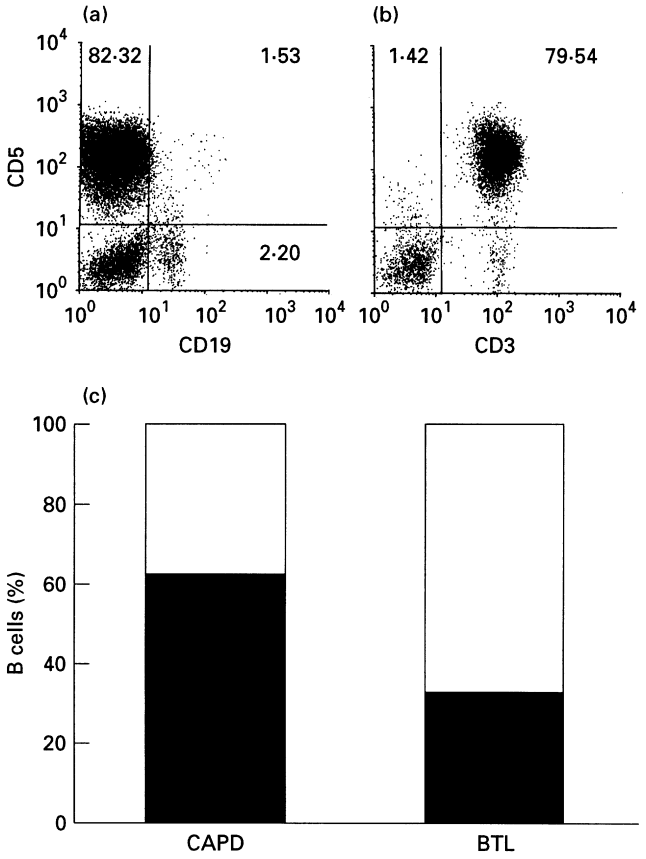

Fig. 1. Two-colour flow cytometry of peritoneal cells. The B-1 cell populations were determined by positive co-staining for CD19 and CD5 surface markers (a) but negative staining for CD3 (b). The B-2 or conventional B cells were $CD19^+$ but $CD5^-$ (a). The composition of the total peritoneal B cell population was determined as the percentage of B-1 (□) and B-2 (■) cells from the peritoneal fluid collected from 11 continuous ambulatory peritoneal dialysis (CAPD) and 11 bilateral tubal ligation (BTL) patients (c).

comprised a large percentage of the peritoneal lymphocytes, while the B cells represented only a minor population recovered from the peritoneal effluent.

To determine the population of B-1 cells within the peritoneal B cell population, two-colour flow cytometry analysis with anti-CD5 and anti-CD19, as well as anti-CD5 and anti-CD3 antibodies, was performed (Fig. 1a,b). From the BTL patients, B-1 cells represented 63% of the B cells; in the CAPD group, B-1 cells accounted for 38% of the B cell population (Fig. 1c). In both groups, B-1 cells comprised ≈ 21·5% of the total peripheral blood B cells.

To characterize further the peritoneal lymphocyte population for expression of potential homing receptors for the mucosal and systemic lymphoid compartments, peritoneal cells from three patients were analysed for expression of L-selectin, $\alpha 4\beta 7$ and $\alpha E\beta 7$ (Table 2). When the peritoneal cells and PBMC from the same individual were compared, the percentage of peritoneal cells expressing the mucosal homing receptors was higher and the PLN receptor lower than in the PBMC. When B-1 cells were examined, most of the peritoneal and peripheral B-1 cells expressed $\alpha 4\beta 7$, $\alpha E\beta 7$, and L-selectin. Immunohistochemical staining showed that ≈ 15% of peritoneal cells were positive for cytoplasmic immunoglobulin. Of these, 43% were IgG^+, 35% were IgM^+, and 22% were IgA^+.

Levels of immunoglobulin in peritoneal effluent and the effect of long-term dialysis on salivary and serum immunoglobulins

Peritoneal fluid contained all three major isotypes of immunoglobulin, although levels were lower than those measured in the corresponding serum sample (Table 3). To determine the approximate loss of immunoglobulin by repeated flushing of the peritoneal cavity during dialysis, we measured the average level of IgG, IgM and IgA in peritoneal fluids, and related these levels to the total volume of fluid collected. The average peritoneal fluid collected (≈2 *l*) contained a total of 286 mg of IgG, 60 mg of IgA, and 14 mg of IgM at the time of initial dialysis. The lowest values for peritoneal immunoglobulin were obtained at 6 months of dialysis, in which the combined average of peritoneal IgG, IgA and IgM was 29 mg. We estimate that between 87 and 1160 mg of

Table 2. Homing receptor expression on peritoneal cells and PBMC

		MNC* (%)			B-1 cells† (%)		
	Patients	$\alpha 4\beta 7$	$\alpha E\beta 7$	CD62L	$\alpha 4\beta 7$	$\alpha E\beta 7$	CD62L
PerC	1	61	41	45	72	71	95
	2	83	43	90	95	97	96
	3	23	16	24	99	86	96
PBMC	1	28	13	68	49	94	69
	2	46	38	64	66	64	85
	3	24	11	80	77	57	95

* The mononuclear cell (MNC) populations from the peritoneal cell recovery (PerC) and PBMC from autologous donors were analysed by FACS for the expression of the mucosal homing receptor, $\alpha 4\beta 7$; the mucosal-associated integrin, $\alpha E\beta 7$; and the peripheral homing receptor, L-selectin (CD62L). Data are expressed as the percentage of the total gated population.

† The homing receptor expression on the B-1 cell population was determined by three-colour FACS. Data are reported as the percentage of B-1 cells expressing each integrin.

Table 3. Peritoneal fluid and serum immunoglobulin levels

	Immunoglobulin source	Volume* (ml)	IgG† (μg/ml)	IgA† (μg/ml)	IgM† (μg/ml)
BTL ($n = 16$)	Peritoneal‡§	10 (10–27)	3233 ± 2951	165·4 ± 90·0	118 ± 96·2
CAPD ($n = 11$)	Peritoneal‡	1898 (1500–2850)	150·5 ± 93·5	32·4 ± 19·2	7·45 ± 4·76
CAPD	Serum immunoglobulin¶		12 593 ± 3759	2318 ± 981	910 ± 238

* Results are expressed as median (range).
† Results are expressed as mean ± s.d.
‡ Immunoglobulin levels were determined by ELISA.
§ Serum was not collected from the bilateral tubal ligation (BTL) patients.
¶ Immunoglobulin levels were measured by nephelometry.
CAPD, Continuous ambulatory peritoneal dialysis.

immunoglobulin were lost in the dialysis effluent, which includes three-to-four dialysis exchanges with ≈ 2 *l* of fluid each on a daily basis. Although these losses of immunoglobulin are considerable, no significant differences were found in the levels of salivary or serum IgA, IgG and IgM levels in long-term patients (Fig. 2). However, levels of immunoglobulin found in peritoneal effluent were reduced over time in each individual (Fig. 2).

Influence of long-term dialysis on peritoneal lymphocytes and PBMC

To determine whether the CAPD influences the total number as well as the composition of cells in the peritoneal effluent and PBMC, three CAPD patients were followed for 1 year; values for peritoneal cells but not PBMC for a fourth patient were available. The total number of peritoneal lymphocytes was reduced by 85%

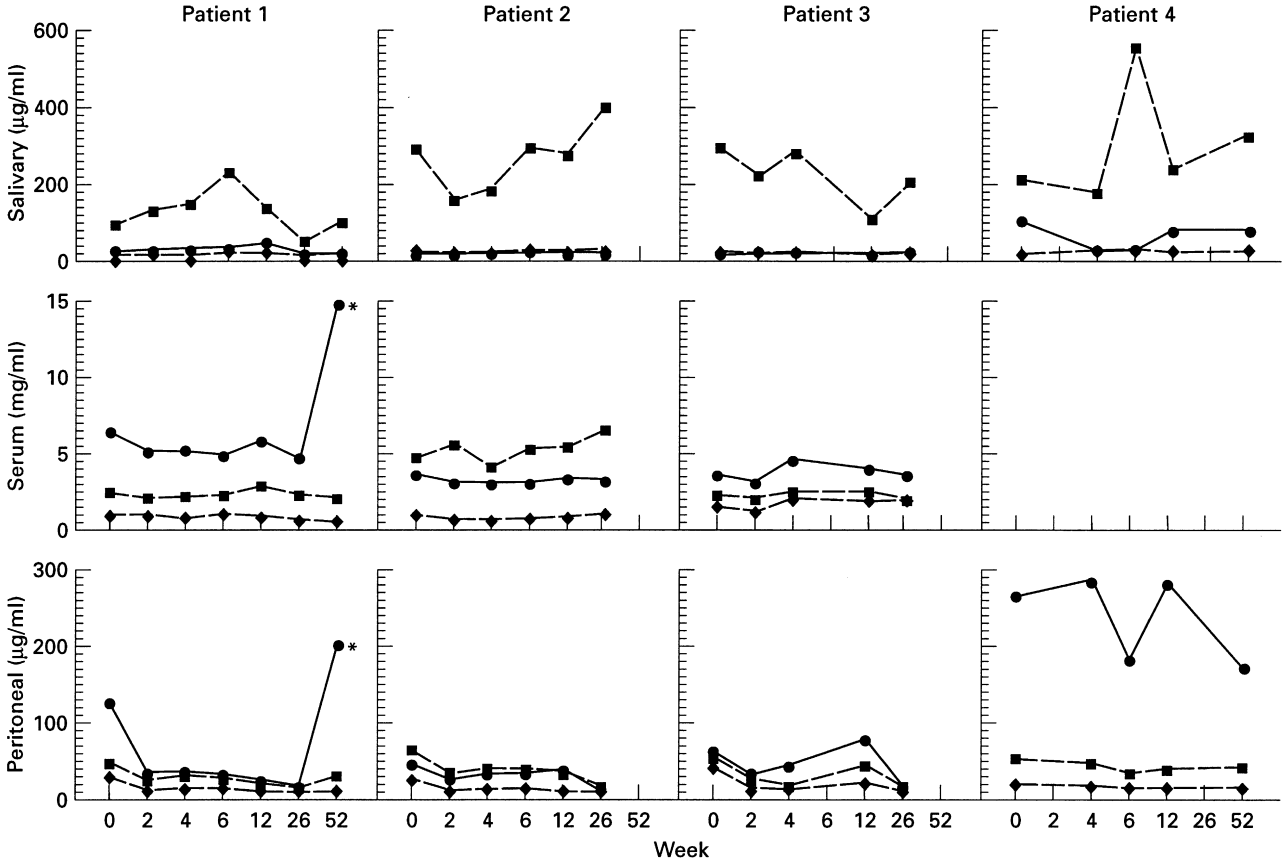

Fig. 2. Immunoglobulin levels in four continuous ambulatory peritoneal dialysis (CAPD) patients from the initial dialysis to 1 year. ELISA was used to quantify IgG (●), IgA (■), and IgM (◆) in the saliva, serum, and peritoneal effluent. *Patient 1 was seronegative for HIV at 52 weeks, but seroconverted within the next 6 months.

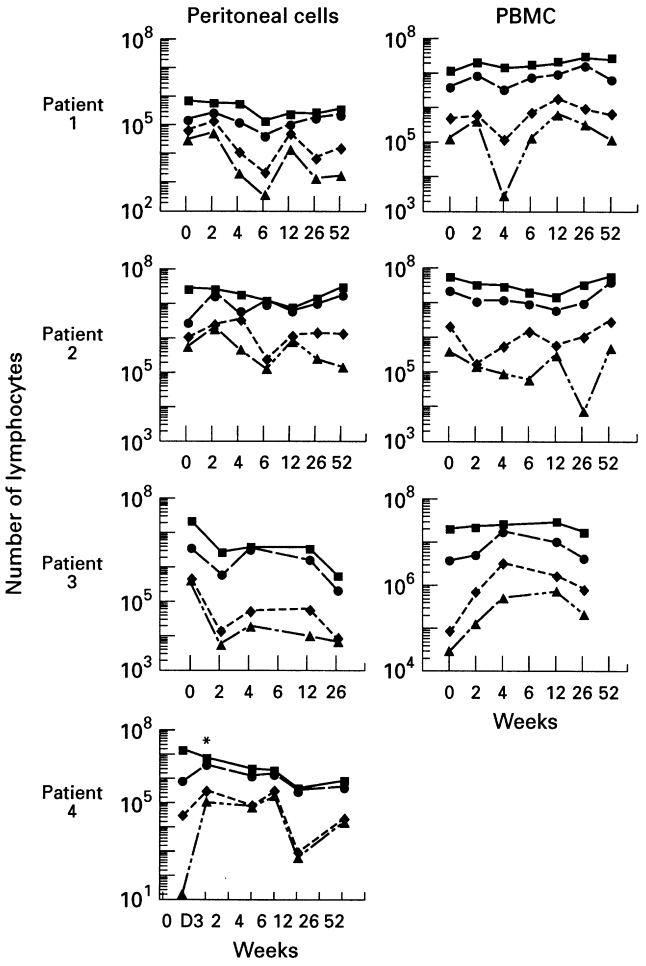

Fig. 3. The phenotype of peritoneal and peripheral blood lymphocyte populations determined by two-colour flow cytometry. The lymphocyte gate was used to determine the number of peritoneal lymphocytes or PBMC (■) over the course of continuous ambulatory peritoneal dialysis (CAPD), beginning with the initial dialysis. The T cell population was identified by staining with anti-CD3 (●); anti-CD19 was used to distinguish the B cells (◆), and B-1 cells expressed both CD19 and CD5 (▲). PBMC from patient 4 were not available. *Patient 4 had very few $CD5^+$ B cells on day 0, but by day 3 the numbers had stabilized.

by CAPD; however, the total number of PBMC was not influenced by the treatment (Fig. 3). T cells, which represent the majority of the peritoneal lymphocyte population, remained relatively constant. The number of peritoneal B cells was reduced by 50%. This decrease in B cell numbers was also reflected in B-1 numbers, indicating little change in the ratio of B-1 to B-2 cells after the first 2 weeks (Fig. 3). In four patients in whom peritoneal effluents were collected on a daily basis for the first week, an increase in B-1 cell numbers was observed. Afterwards the ratio of B-1 to B-2 remained constant (data not shown).

DISCUSSION

Human peritoneal lymphocyte populations have been characterized in a limited number of studies [20,26–31]. These studies have shown that human peritoneal fluid contains relatively few lymphocytes. The cells analysed in this study should be representative of the phenotype of the normal peritoneal lymphocyte population, because the patients did not have clinical signs of inflammation such as peritonitis or endometriosis, as reflected by the low PMN counts.

In our previous work, we analysed cell populations from patients who had been on CAPD for over 2 years [20,21]. Therefore in this study, we determined the cell composition immediately after initiation of CAPD and in patients undergoing BTL. In addition, we were able to evaluate the influence of long-term CAPD treatment on the level of serum and salivary immunoglobulin levels, as well as the peripheral and peritoneal lymphocyte populations from four CAPD patients. In contrast to mice, the extensive drainage of the peritoneal lymphocyte populations did not substantially alter the levels of immunoglobulin in serum or saliva, nor did it influence the lymphocyte population in peripheral blood. Although B-1 cells comprise a large percentage of the human and mouse peritoneal B cell population, their depletion from the peritoneal cavity resulted in profound decreases in serum IgM levels in mice [13], but did not appear to result in an analogous change in humans. This suggests that human peritoneal B-1 cells, that contribute to the serum IgM pool, contribute only a minute fraction, and thus their loss does not translate into changes in serum IgM levels.

By calculating the amount of IgA, IgM and IgG in the total daily peritoneal effluent, we estimated that 2–27% of immunoglobulin synthesized daily [32] in an average 70-kg person will be removed through flushing the peritoneal cavity. The loss of immunoglobulin was not reflected in decreased levels of serum immunoglobulin, and presumably was compensated for by either an increase in production or a decrease in catabolism of immunoglobulin.

Another distinction between humans and mice is the contribution of the peritoneal B-1 cell population to the mucosal compartment of the immune system. In mice, peritoneal B-1 cells appear to contain a subset of precursors of the IgA plasma cells that have the potential to migrate to the intestinal lamina propria [3,33]. Most peritoneal lymphocytes express $\alpha 4$ and $\beta 7$ in mice (Fengyu Shu, UAB, Birmingham, AL, personal communication), which is indicative of mucosal precursor cells [34–36]. Whether human lymphocytes from the peritoneal cavity can traffic to the gut is still unresolved. Human peritoneal lymphocytes differ from PBMC in the expression of mucosal homing receptors; however, homing receptors for both PLN and mucosa were expressed on human peritoneal B-1 cells. Based on this phenotype alone, human peritoneal lymphocytes should have the potential to home to mucosal sites [34,36], but this is difficult to prove experimentally in humans.

The human peritoneal B-1 cell population is comparable to its murine counterpart based on phenotypic analysis. However, the contributions by the peritoneal B-1 cell to both the systemic and mucosal immune compartments appear to differ between mice and humans. This may reflect the experimental approaches available to study these populations in humans.

ACKNOWLEDGMENTS

The authors thank the nurses at the UAB home training dialysis unit: especially Barbara Hubbard, Sarah Kennedy, Kim Rowley, and Michelle Jackson, and Dr Brian Gleason, Department of Medical Surgical Gynaecology, for providing peritoneal samples. We also would like to thank Shirley Prince and Rose Kulhavy for their technical assistance. Thanks to Drs John Kearney and Charles Elson for kindly reviewing the manuscript,

and to the UAB Centre for AIDS Research Flow Cytometry Facility. This work was supported by grants AI07051, DK49358, HD33168, DE08182, and AI28147.

REFERENCES

1 Hayakawa K, Hardy RR, Herzenberg LA. Peritoneal Ly-1 B cells: genetic control, autoantibody production, increased lambda light chain expression. Eur J Immunol 1986; **16**:450–6.

2 Hayakawa K, Hardy RR, Parks DR, Herzenberg LA. The 'Ly-1 B' cell subpopulation in normal immunodefective and autoimmune mice. J Exp Med 1983; **157**:202–18.

3 Kroese FGM, Butcher EC, Stall AM, Herzenberg LA. A major peritoneal reservoir of precursors for intestinal IgA plasma cells. Immunol Invest 1989; **18**:47–58.

4 Pecquet SS, Ehrat C, Ernst PB. Enhancement of mucosal antibody responses to *Salmonella typhimurium* and the microbial hapten phosphorylcholine in mice with X-linked immunodeficiency by B-cell precursors from the peritoneal cavity. Infect Immun 1992; **60**:503–9.

5 Herzenberg LA, Kantor AB. B-cell lineages exist in the mouse. Immunol Today 1993; **14**:79–83.

6 Kantor AB. The development and repertoire of B-1 cells (CD5 B cells). Immunol Today 1991; **12**:389–91.

7 Kasaian MT, Ikematsu H, Casali P. $CD5^+$ B lymphocytes. Proc Soc Exp Biol Med 1991; **197**:226–41.

8 Kipps TJ. The CD5 B cells. Adv Immunol 1989; **47**:117–85.

9 Kroese FGM, Butcher EC, Stall AM *et al*. Many of the IgA producing plasma cells in murine gut are derived from self-replenishing precursors in the peritoneal cavity. Int Immunol 1989; **1**:75–84.

10 Kroese FGM, Ammerlaan WAM, Kantor AB. Evidence that intestinal IgA plasma cells in μ, κ transgenic mice are derived from B-1 (Ly-1 B) cells. Int Immunol 1993; **5**:1317–27.

11 Beagley KW, Bao S, Ramsay AJ, Eldridge JH, Husband AJ. IgA production by peritoneal cavity B cells is IL-6 independent: implications for intestinal IgA responses. Eur J Immunol 1995; **25**:2123–6.

12 Bos NA, Bun JCAM, Popma SH *et al*. Monoclonal immunoglobulin A derived from peritoneal B cells is encoded by both germ line and somatically mutated V_H genes and is reactive with commensal bacteria. Infect Immun 1996; **64**:616–23.

13 Marcos MAR, Gaspar ML, Malenchere E, Coutinho A. Isolation of peritoneal precursors of B-1 cells in the adult mouse. Eur J Immunol 1994; **24**:1033–40.

14 Hiroshi I, Hastings R, Kearney J, Howard M. Continuous anti- interleukin 10 antibody administration depletes mice of Ly-1 B cells but not conventional B cells. J Exp Med 1992; **175**:1213–20.

15 Kipps TJ, Vaughan JH. Genetic influence on the levels of circulating CD5 B lymphocytes. J Immunol 1987; **139**:1060–4.

16 Hardy RR, Hayakawa K, Shimizu M *et al*. Rheumatoid factor secretion from human Leu-1 B cells. Science 1987; **236**:81–83.

17 Solvason N, Kearney JF. The human fetal omentum: a site of B cell generation. J Exp Med 1992; **175**:397–404.

18 Bhat NM, Kantor AB, Bieber MM *et al*. The ontogeny and functional characteristics of human B-1 ($CD5^+$ B) cells. Int Immunol 1992; **4**:243–52.

19 Casali P, Notkins AL. $CD5^+$ B lymphocytes, polyreactive antibodies and the human B-cell repertoire. Immunol Today 1989; **10**:364–8.

20 Lue C, Van den Wall Bake AWL, Prince SJ *et al*. Intraperitoneal immunization of human subjects with tetanus toxoid induces specific antibody-secreting cells in the peritoneal cavity and in the circulation, but fails to elicit a secretory IgA response. Clin Exp Immunol 1994; **96**:356–63.

21 Lue C, van den Wall Bake AML, Prince SJ *et al*. Intraperitoneal administration of tetanus toxoid elicits a specific response of antibody-secreting cells in the peritoneal cavity. Adv Exp Med Biol 1995; **317A**:103–6.

22 Popovich RP, Moncrief JW, Nolph KD *et al*. Continuous ambulatory peritoneal dialysis. Ann Intern Med 1978; **88**:449–56.

23 Lazarovitis AI, Moscicki R, Kurnick J *et al*. Lymphocyte activation antigens. I. A monoclonal antibody anti-Act-1, defines a new late lymphocyte activation antigen. J Immunol 1984; **133**:1857–62.

24 Mestecky J, Moldoveanu Z, Julian BA, Prchal JT. J chain disease: a novel form of plasma cell dyscrasia. Am J Med 1990; **88**:411–6.

25 Rose NR, Friedman H, Fahey JL. Manual of clinical laboratory immunology, 3rd edn. Washington, DC: American Society for Microbiology, 1986.

26 Yoffey JM. Peritoneal lymphoid cells. Lymphology 1986; **19**:96–105.

27 Rubin J, Lin LM, Lewis R *et al*. Host defense mechanisms in continuous ambulatory peritoneal dialysis. Clin Nephrol 1983; **20**:140–4.

28 Brando B, Galato R, Seveso M *et al*. Flow cytometric study of immunocompetent cell phenotypes and phagocytosis in CAPD effluent. Trans Am Soc Artif Intern Organs 1988; **34**:441–4.

29 Kubicka U, Olszewski WL, Maldyk J *et al*. Normal human peritoneal cells: phenotypic characteristics. Immunobiology 1989; **180**:80–92.

30 Davies SJ, Suassuna J, Ogg CS, Cameron JS. Activation of immunocompetent cells in the peritoneum of patients treated with CAPD. Kidney Int 1989; **36**:661–8.

31 Hill JA, Faris HMP, Schiff I, Anderson D. Characterization of leukocyte subpopulations in the peritoneal fluid of women with endometriosis. Fertil Steril 1988; **50**:216–22.

32 Hasemann CA, Capra JD. Immunoglobulins: structure and function. In: Paul WE, ed. Fundamental immunology. New York: Raven Press, 1989:209–33.

33 Murakami M, Honjo T. Involvement of B-1 cells in mucosal immunity and autoimmunity. Immunol Today 1995; **16**:534–9.

34 Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. Science 1996; **272**:60–66.

35 Weissman IL. From thymic lineages back to hematopoietic stem cells, sometimes using homing receptors. J Immunol 1996; **156**: 2019–25.

36 Wagner N, Löhler J, Kunkel EJ *et al*. Critical role for β7 integrins in formation of the gut-associated lymphoid tissue. Nature 1996; **382**: 366–70.