Recurrent Pyogenic Cholangitis: Is Immune Depression a Feature ?

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Running Title:

Immune deficit in recurrent pyogenic cholangitis

Abstract

As the name suggests, patients with recurrent pyogenic cholangitis (RPC) are prone to repeated episodes of cholangitis with systemic sepsis. The mechanism and pathogenesis of sepsis in these patients is not well understood, although some animal studies have suggested a functional defect in the immune system. To determine whether an immunological deficit may account for RPC, we conducted a pilot study to assess the immune status of these patients and compared it to a control group. Using two-colour flow cytometry, we found that the absolute number of various lymphocytes subpopulations, namely total T cells (CD3+), CD4+ T cells, total B cells (CD19+) and natural killer (NK) cells (CD16+ 56+) seemed to be depressed in patients with RPC as compared to the control group. In addition we observed that these patients had higher levels of CD8+ cells expressing the CD38 activation marker. Although based on a small sample size, these findings suggest that immune depression may be an important feature of RPC.

Keywords:

Recurrent Biliary Sepsis, Primary Calculi, Immune Depression

Introduction.

Patients with biliary obstruction are more susceptible to septic complications. Unlike the West, patients with recurrent pyogenic cholangitis (RPC) in South East Asia do not have complete obstruction of the bile ducts but tend to have patulous papillae and adequate bile flow [1]. They experience repeated invasion of the biliary tract by gut organisms and develop soft primary bilirubinate calculi within dilated ducts from bacterial degradation of bile[2]. The literature however, has not provided an adequate explanation for the aetiology of this disease. A number of studies carried out in animals have demonstrated a functional depression of the immune system following obstructive jaundice [3,4,5]. For example, depression of the reticulo-endothelial system and T cell dysfunction have been reported in rats with obstructive jaundice which appears to prolong allograft survival in these animals [4,5,6].

Possible immune modulation of patients with RPC has not been addressed. As a preliminary approach, we assessed the immunological status of a small group of patients with RPC by determining the percentages and the absolute numbers of various peripheral blood lymphocyte subsets as well as their activation markers using two-colour flow cytometry. The lymphocyte subsets measured were T, B and NK cells as well as the T lymphocyte subsets, CD4+, CD8+ and $\gamma\delta$+ cells. Comparison was made with a control group, comprising of patients undergoing elective surgery and those with other inflammatory diseases as well as with previous data obtained from normal individuals.

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Materials and Methods

a) Subjects

Five patients with cholangitis and six patients undergoing elective surgery or with other intra-abdominal infections were included in this study. All those included were patients of Hospital Universiti Sains Malaysia.

b. Sample Preparation:

Blood specimens were collected in sterile tubes containing anti-coagulant. High resolution two-colour flow cytometric analysis was performed on peripheral blood samples prepared using standardised lysed whole blood method [7]. Briefly, 100 µl of whole blood samples were placed into a set of polystyrene tubes containing 10 µl of matching combinations of murine monoclonal antibodies directly conjugated with fluorescein isothiocynate and phycoerythrin (Becton Dickinson). The test monoclonal antibodies used and their specificities is shown in Table 1. The samples were then incubated in the dark for 15 min at room temperature after which the erythrocytes were lysed in 2 ml of FACSLysing™ solution. The samples were washed once in phosphate-buffered saline, fixed in 1% paraformaldehyde and 10,000 cells were analysed in the FACScan™ using the SimulSET™ Software (Becton Dickinson) as previously described [8].

c. Statistical Analyses:

The number of specimens studied was too small for any accurate statistical analysis to be performed. However, to provide some indication of the differences in various lymphocyte populations studied in patients with RPC and surgical/inflammatory conditions, an unpaired Student t-test was carried out . Comparisons with those of normal individuals were also included. The statistical analysis was carried out using the StatWorks™ software (Apple Computer, Inc.).

Results

Table 2 shows the percentage levels and absolute numbers of various lymphocyte subsets in patients with RPC as compared to control groups. As observed, the total percentage of lymphocytes in RPC was lower than controls. The percentages of subpopulations of lymphocytes remained the same. However, there was an apparent reduction in the absolute number of total T cells (CD3+) and CD4+ T cells as well as total B cells (CD19+) and NK cells (CD16+56+). No apparent difference in the absoluate numbers of CD8+ T cells and γδ+ T cells were observed. The percentage and absolute number of lymphocyte subpopulations in the surgical and inflammatory controls were similar to normal controls.

Table 3 shows the percentages of CD4+, CD8+ and γδ+ T cell subsets expressing selected activation markers. There was no remarkable difference between the RPC and control groups. However, an apparent increase in the number of CD8+ T cells expressing the CD38 activation marker was observed in patients with RPC.

Discussion

Classically, cholangitis results from biliary obstruction caused by calculi. However, since RPC is primarily a bacterial cholangitis, recurrent biliary tract infection by gut organisms is also seen in the absence of calculi and obstruction. To investigate this recurrent biliary infection, we focused our attention on the possibility of immune incompetence as an explanation for the repeated systemic and biliary tract bacterial colonization.

We found that the percentage of total lymphocytes were reduced in patients with RPC as compared to control groups. However, the percentage levels of the various lymphocyte subsets were unchanged. Thus assessing immune competence in these patients by CD4:CD8 ratio or percentage levels alone would not provide an accurate indication of the immune status since they would appear normal. Therefore we also determined the absolute numbers of the various lymphocyte subsets. We found an apparent reduction in the total numbers of T cells, B cells and NK cells which suggest an overall immune depression in these patients. Of relative importance was the reduction in CD4+ T cells because these cells play a pivotal role in driving both arms of the immune response by the production of lymphokines. CD4+ T cell reduction has been documented in several immune deficiency states like AIDS [10] and it is quite likely that the susceptibility to repeated sepsis in RPC is also due a reduction in these cells.

With regard to the activation status of T lymphocytes, we did not observe any remarkable difference between RPC and the control group. However, CD8+ T cells expressing CD38 was proportionately higher than that of the control group. This was reminiscent to previous studies in HIV-infection, where strong correlation between increase in CD8+ T cells expressing CD38 and decline of immunocompetence has been reported [8,11]. This further suggests that immune depression may be a common feature of RPC.

This study was based on a very small sample size which was due to the strict inclusion

criteria used. The samples were processed only if freshly drawn or transported within 2 hours of collection without refrigeration and before beginning antibiotic-treatment. Several samples obtained from peripheral hospitals were discarded because of non-adherence to these criteria. The surgical and inflammatory controls were grouped and analysed together because of the small numbers involved and thus possible differences between these individuals could not be ascertained. However, a pattern of immune depression could be observed and ~~thus~~ further studies need to be carried out to substantiate the present findings. Such studies should include functional assessment of T cells as well as the measurements of other immune parameters such as immunoglobulin and complement levels.

Table 1:

Panel of mouse monoclonal antibody used (Becton Dickinson).

	monoclonal antibody	Specificity
		Lymphocyte Subpopulation
1	CD3	Total T lymphocytes
2	CD4	T helper subset
3	CD8	T cytotoxic/suppressor subset
4	γδ	T lymphocytes expressing the γδ TCR
5	CD19	Total B lymphocytes
6	CD16+56	Most NK cells
		T Lymphocyte Activation Markers*
7	CD25	Low affinity IL2-receptor
8	HLA-DR	MHC class II antigen
9	CD38	Activated/immature lymphocyte

* The T lymphocyte activation markers were each assessed on all the T cell subpopulation, namely, CD4, CD8 and γδ cells. Assessment of the expreesion of CD38 on CD4 cells was not carried out (see Table 3)

Table 2:

Percentage level and absolute number of various lymphocyte subsets (mean ± s.d.).

Cell Type	Normal Controls [a]	Reccurent pyogenic cholangitis	Surgical and Inflammatory Controls	p values [b]
Total percentage lymphocytes	30.0 ± 8.7 (32) [c]	14.4 ± 6.1 (5)	24.3 ± 6.4 (6)	0.028
CD3%	68.9 ± 9.7 (32)	62.0 ± 5.7 (5)	61.7 ± 2.6 (6)	-
CD3 absolute number (10^3/ml)	1.62 ± 0.55 (32)	0.84 ± 0.35 (5)	1.99 ± 0.77 (6)	0.014
CD4%	35.1 ± 6.7 (50)	34.8 ± 5.4 (5)	32.5 ± 8.6 (6)	-
CD4 absolute number (10^3/ml)	0.76 ± 0.30 (50)	0.46 ± 0.15 (5)	0.97 ± 0.10 (6)	< 0.0001
CD8%	29.3 ± 8.4 (50)	24.6 ± 5.4 (5)	27.2 ± 9.0 (6)	-
CD8 absolute number (10^3/ml)	0.64 ± 0.31 (50)	0.34 ± 0.17 (5)	0.93 ± 0.62 (6)	-
γδ%	6.9 ± 5.5 (40)	4.4 ± 3.3 (4)	3.0 ± 2.2 (3)	-
γδ absolute number (10^3/ml)	0.18 ± 0.17 (40)	0.05 ± 0.02 (4)	0.08 ± 0.06 (3)	-
CD19%	13.2 ± 4.1 (32)	15.2 ± 8.6 (5)	14.0 ± 4.2 (6)	-
CD19 absolute number (10^3/ml)	0.31 ± 0.14 (32)	0.18 ± 0.08 (5)	0.44 ± 0.17 (6)	0.013
CD16+56%	19.3 ± 9.7 (32)	22.6 ± 11.9 (5)	22.7 ± 6.7 (6)	-
CD16+56 absolute number (10^3/ml)	0.46 ± 0.31 (32)	0.33 ± 0.23 (5)	0.68 ± 0.17 (6)	0.02

[a] = from [8,9]

[b] p values are based on unpaired Student t-test between patients with RPC and surgical/inflammatory controls only. p values of >0.05 are not shown

[c] = numbers in parentheses indicate the number of specimens.

Table 3:

Expression of activation and memory markers on various T lymphocyte subsets (mean ± s.d.).

Marker	Normal Controls [a]	Reccurent pyogenic cholangitis	Surgical and Inflammatory Controls	p values [b]
CD4+ lymphocytes expressing				
CD25	39.1 ± 9.0 (37) [c]	45.9 ± 15.2 (4)	52.8 ± 9.5 (6)	-
HLA-DR	19.0 ± 8.6 (37)	31.9 ± 12.3 (4)	25.0 ± 7.9 (6)	-
CD8+ lymphocytes expressing				
CD25	8.0 ± 6.0 (22)	10.9 ± 5.6 (3)	11.6 ± 4.3 (6)	-
HLA-DR	44.2 ± 14.0 (34)	70.9 ± 4.2 (3)	69.1 ± 19.0 (6)	-
CD38	73.3 ± 10.4 (34)	81.3 ± 5.6 (3)	61.7 ± 9.4 (4)	0.025
γδ+ lymphocytes expressing				
CD25	4.6 ± 11.3 (21)	0 (3)	38.9 ± 34.7 (3)	-
HLA-DR	59.4 ± 15.9 (24)	84.5 ± 18.6 (4)	75.0 ± 25.0 (3)	-
CD38	52.6 ± 24.5 (27)	77.0 ± 20.6 (4)	26.7 ± 46.2 (3)	-

[a] = from [8].

[b] p values are based on unpaired Student t-test between recurrent pyogenic cholangitis and surgical/inflammatory controls only. p values of >0.05 are not shown

[c] = numbers in parentheses indicate the number of specimens.

References

1 Toufeeq Khan TF, Lwin M, Ulah S, Zahari A, Mokti I. Bilioenteric anastomosis; Results in benign and malignant conditions. Sing Med J 1993;34:545-50.

2 Lam SK, Wong KP, Chan PKW, Ngan H, Ong GB. Recurrent Pyogenic Cholangitis: A study by endoscopic retrograde cholangiography. Gastroenterology 1978;74:1196-203.

3 Holman JL, Rikkers L. Biliary obstruction and host defence failure. J Surg Res 1982;32:208-13.

4 Tanaka N, Ryden S, Bergquist L, Christensen P, Bengmark S. Reticuloendothelial function in rats with obstructive jaundice. Br J Surg 1985;72:946-9.

5 Ding DW, Andersson R, Stenram U, Lunderquist A, Bengmark S. Effect of biliary decompression on reticuloendothelial function in jaundiced rats. Br J Surg 1992; 79:648-52.

6 Rougheen PT, Kulkarni AD, Gouma DJ, Fanslow WC, Rowlands BJ. Suppression of host-versus-graft response in experimental biliary obstruction. Transplantation 1986;42:687-9.

7 Loken MR, Brosnan JM, Bach BA, Ault KA. Establishing optimal lymphocyte gates for immunophenotyping by flow cytometry. Cytometry 1990;11: 453 - 59.

8 Norazmi MN, Jamaruddin MA, Arifin H. Expression of the T lymphocyte activation markers, CD25, HLA-DR, CD38 and memory cell marker CD45RO on the CD4+, CD8+ and γδ+ lymphocyte subsets in HIV-infected intravenous drug users. AsP J Mol Biol Biotech 1995;3: 119 - 29.

9 Norazmi MN, Suarn S. Disparity in the percentage of CD4+ T lymphocytes and prognosis of HIV-infected intravenous drug users in Malaysia. Immunol Lett 1994;43, 177 - 82.

10 Fahey JL, Prince H, Weaver M, *et. al.* Quantitative changes in T helper or T suppressor/cytotoxic lymphocyte subsets that distinguish Acquired Immune Deficiency Syndrome from other immune subset disorders. Am J Med 1984;76, 95 - 100.

11 Kestens L, Vanham G, Gigase P *et al.* Expression of activation antigens, HLA-DR and CD38, on CD8 lymphocytes during HIV-1 infection. AIDS 1992;6: 793 - 97.

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Laporan akhir projek penyelidikan "Profil Imunologi Dalam Kolangitis Berulang", Prof. Madya T.F. Taufeeq Khan (Penyelidik Utama), Disember 1993 ke November 1995

Merujuk kepada perkara di atas, saya sebagai penyelidik bersama projek tersebut dengan ini lampirkan penerbitan kami hasil daripada penyelidikan tersebut.

Melalui kesempatan ini, saya dan Dr. Taufeeq Khan ingin merakamkan penghargaan kami di atas peruntukan geran jangka pendek tersebut.

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Yang benar,

.....

Prof. Madya Norazmi Mohd. Nor
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Pusat Pengajian Sains Perubatan

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Recurrent pyogenic cholangitis: is immune depression a feature?

Classically, cholangitis results from biliary obstruction caused by calculi. However, patients with recurrent pyogenic cholangitis (RPC) are prone to repeated episodes of cholangitis with systemic sepsis in the absence of complete obstruction of the bile ducts[1]. The mechanism and pathogenesis of sepsis in these patients is not well understood. Although a number of animal studies have demonstrated a functional depression of the immune system following obstructive jaundice[2,3], a possible immune modulation of patients with RPC has not been addressed.

To determine whether an immunological depression is a feature of RPC, we conducted a preliminary study to assess the levels of various lymphocyte subsets (T, B, NK cells) as well as the various T cell subsets (CD4+, CD8+ and γδ cells). We used dual-colour flow cytometry, in a small group of patients with RPC ($n=5$) and compared it to a control group comprising of two patients with acute appendicitis, one with peritonitis and three patients undergoing elective surgery.

The total white cell count of patients in the control group was slightly higher than normal, probably due to the neutrophilia observed. That of the RPC group was within normal range, despite similar levels of neutrophilia. This may be due to the marked lymphopenia in the latter group. The percentage of total lymphocytes dropped by approximately 50% from normal values to 14.4% of total white cells in patients with RPC. There was a proportionate reduction in all the various lymphocyte subsets including those of the T cell subsets in these patients. However, we observed that the absolute number of CD4+T cells was greatly diminished in the RPC group (460 ± 150 cells per microlitre) as compared to controls (970 ± 100 cells per microlitre) ($P<0.0001$, Student's t test) while those of other T cell subsets were not significantly reduced. This observation may be important because CD4+T cells play a pivotal role in driving both arms of the immune response by the production of lymphokines. Absolute CD4+T cell lymphopenia has been documented in several immune deficiency states like in the advanced stages of HIV-infection and AIDS[4]. It is quite likely that the susceptibility to repeated sepsis in RPC may also be due to a reduction in these cells. In addition, as with HIV-infection[5], we observed that a significant level of CD8+T cells in the RPC group was activated by virtue of their expression of the CD38 marker as compared to the control group ($P<0.03$). The increased level of activated CD8+T cells in HIV-infection has been attributed to their chronic stimulation towards viral infected cells[6]. The significance of this observation in RPC is however uncertain.

Although based on a small sample size, the finding that patients with RPC have marked lymphopenia with significant reduction in the number of CD4+T cells may suggest that immune depression may be an important feature of RPC. Whether this is a primary or secondary feature of RPC remains to be investigated. Obviously, further studies need to be carried out to substantiate the present findings. Such studies should include functional assessment of T cells as well as the measurements of other immune parameters such as immunoglobulin and complement levels.

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REFERENCES

1 Toufeeq Khan TF, Lwin M, Ulah S, Zahari A, Mokti I. Bilioenteric anastomosis; Results in benign and malignant conditions. *Sing Med J* 1993;34:545–50
2 Holman JL, Rikkers L. Biliary obstruction and host defence failure. *J Surg Res* 1982;32:208–13
3 Ding DW, Andersson R, Stenram U, Lunderquist A, Bengmark S. Effect of biliary decompression on reticuloendothelial function in jaundiced rats. *Br J Surg* 1992;79:648–52
4 Fahey JL, Prince H, Weaver M, Groopman J, Visscher B, Schwart K., *et al.* Quantitative changes in T helper or T suppressor/cytotoxic lymphocyte subsets that distinguish acquired immune deficiency syndrome from other immune subset disorders. *Am J Med* 1984;76:95–100
5 Kestens L, Vanham G, Gigase P, Young G, Hannet L, Vanlangendonck F, *et al.* Expression of activation antigens, HLA-DR and CD38, on CD8 lymphocytes during HIV-1 infection. *AIDS* 1992;6:793–7
6 Ho H-N, Hultin LE, Mitsuyasu RT, Matud JL, Hausner MA, Bockstoce D, *et al.* Circulating HIV-specific CD8+ cytotoxic T cells express CD38 and HLA-DR antigens. *J Immunol* 1993;150:3070–9

Post-operative fluids

Providing a supply of post-operative fluids has been a personal concern since coming to work in a bush hospital in Congo (ex-Zaire). Our intravenous fluids are flown to us from 1000km away, and this and other costs make the fluids approx £2.50 per litre, adding to our total hospital bill which is already very high in an area where the majority of our patients are subsistence farmers. I have, therefore, been looking at a way of reducing the costs of intravenous fluids.