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The Effect of Anaesthesia and Surgery for Benign Disease of the Upper Urinary Tract on Circulating Leucocyte Subpopulations Identified with Monoclonal Antibodies*

A. W. S. Ritchie, K. James, and G. D. Chisholm

Department of Surgery, University of Edinburgh Medical School, Teviot Place, Edinburgh, UK

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Summary. Circulating lymphocyte subpopulations were monitored, using monoclonal antibodies and flow cytometry, in six patients undergoing surgery for benign disease of the upper urinary tract. A significant decrease in the total number of circulating lymphocytes was observed. This could be attributed to a significant decrease of T cells of both major subsets – the so-called T "helper" (Th) and T "suppressor/cytotoxic" (Ts) subpopulations. When the results of the T cell subsets were expressed as a ratio (leu-$3a^+$/leu-$2a^+$, T "helper/suppressor") no significant change was noted. In contrast neither B cells nor natural killer (NK) and antibody dependent killer (K) cells were significantly affected. This selective loss of T cells from the circulation may be relevant to post operative infection and should be considered in the course of immunological monitoring.

Key words: Anaesthesia, Lymphocyte subsets, Monoclonal antibodies, Flow cytometry.

Introduction

Lymphocytes occupy a central role in the regulation of the immune response and the effects of anaesthesia and surgery on total lymphocytes, T cells (as defined by erythrocyte rosette formation) and B cells are well documented [11]. Recently the advent of monoclonal antibodies [7] together with the development of sophisticated flow cytometry techniques [2] have allowed the examination, both in greater detail and accuracy, of the immunological consequence of anaesthesia and surgery. In this preliminary communication we have used this new technology to investigate the effect of anaesthesia and surgery in patients with benign disease of the upper urinary tract, on circulating T cell subpopulations, B cells and natural killer cells. Such studies we believe are a prerequisite for similar investigations in patients with malignant urological diseases requiring major surgical intervention with the even greater risk of infection.

Patients and Methods

Six patients, 2 male and 4 female, admitted for surgery for benign disease of the upper urinary tract were studied. A control population consisted of 22, age and sex matched helathy individuals. Details of the patients are shown in Table 1.

Blood samples (15 ml) were taken, using EDTA as anticoagulant, between 8am and 9am, just prior to surgery (but before pre-medication) and on postoperative days, 1, 2, 4 and 7. Mononuclear cells were isolated on Ficoll Hypaque (Flow Laboratories, Irvine, Scotland) using the same centrifuge for every specimen. Cells harvested from the interface were washed twice in calcium and magnesium free Hanks balanced salt solution (Gibco Europe) and resuspended in RPMI-1640, supplemented with 10% fetal calf serum and 0.1% sodium azide. Cell viability was assessed using an acridine orange/ethidium bromide mixture [4] and was always greater than 95%. Aliquots of 5×10^5 mononuclear cells were incubated with saturating quantities (determined by titration) of monoclonal antibodies against differentiation antigens on all T cells, the two major T cell subsets $HLADR^+$ cells and NK and K cells. The monoclonal antibodies used were purchased from Becton Dickinson Laboratory Systems, Mechelen, Belgium, with the exception of HNK-1 which was the generous gift of Drs T. Abo and C. M. Balch. Cells binding unconjugated antibodies (HNK-1) were visualised using F $(ab')_2$ sheep anti-mouse Ig fluorescein conjugate. The remaining antibodies were directly conjugated with fluorescein. Cells were then analysed unfixed, using a fluorescence activated cell sorter (FACS IV, Becton Dickinson FACS Systems Sunnyvale, California). Further details of the staining procedure and FACS analysis, including 90° scatter gating, have been described before [8]. Percentage figures obtained from the FACS, based on a count of 10^4 lymphocytes, were used in conjunction with a 200 cell differential count (all performed by one observer) and the total white cell count, to calculate absolute numbers of cells per ml of blood.

Statistical analyses were performed using Friedmans two-way analysis of variance by ranks [10]. For illustrative purpose the mean values ± S.E.M. are given.

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Table 1. Details of the six patients

Patient	Age	Sex	Diagnosis	Operation	Duration of anaesthesia (h)	Postoperative complications
1	52	F	PUJ	Pyeloplasty	1.5	None
2	75	F	Congenital Hydronephrosis	Nephrectomy	1	UTI
3	71	F	Staghorn Calculus	Pyelolithotomy	2	UTI
4	24	F	Non-functioning[a] Kidney	Nephrectomy	1	Wound Infection
5	18	M	PUJ	Pyeloplasty	1	None
6	84	M	Non-functioning[a] Kidney	Nephrectomy	2	None

[a] Both secondary to chronic obstructive uropathy
PUJ = Pelvi-ureteric junction obstruction
All patients had sterile urine culture prior to surgery

Table 2. Sequential analyses of total white cells, lymphocytes and subpopulations

Cell population	Pre-op	Post-op				Xr^2	Statistical significance[b]
		day 1	day 2	day 4	day 7		
Leucocytes x 10^6 per ml							
Total WCC	5.4 ± 0.7[a]	8.6 ± 1.2	8.3 ± 0.8	5.8 ± 0.7	5.7 ± 0.8	18.1	$p < 0.01$
Granulocytes	3.0 ± 0.6	6.8 ± 1.1	6.6 ± 0.8	4.2 ± 0.8	3.1 ± 0.4	17.6	$p < 0.01$
Lymphocytes x 10^5 per ml							
Total lymphocytes	19 ± 2.4	13 ± 1.6	12 ± 2.0	11 ± 2.0	19 ± 3.3	13.1	$p < 0.05$
Leu-4^+ – all peripheral T cells	12.2 ± 2.0	7.6 ± 1.7	6.3 ± 1.0	6.2 ± 1.4	12.0 ± 2.7	12.9	$p < 0.02$
Leu-$3a^+$ – helper T cells	8.3 ± 1.3	5.0 ± 1.1	4.1 ± 0.6	4.4 ± 1.0	9.2 ± 2.2	13.2	$p < 0.01$
Leu-$2a^+$ – suppressor/cytotoxic T cells	3.7 ± 1.0	2.2 ± 0.6	2.2 ± 0.6	2.0 ± 0.6	3.3 ± 0.9	9.9	$p < 0.05$
$HLADR^+$[c] B cells and activated T cells	3.9 ± 0.6	3.3 ± 0.6	2.3 ± 0.3	2.8 ± 0.6	4.6 ± 0.6	7.3	N.S.
HNK-1^+ NK and K cells	2.7 ± 0.4	2.2 ± 0.4	2.0 ± 0.3	1.7 ± 0.3	2.5 ± 0.6	1.7	N.S.
Leu-$3a^+$/Leu-$2a^+$ ratio	2.4 ± 0.4	2.1 ± 0.2	1.9 ± 0.3	2.4 ± 0.6	3.0 ± 0.6	4.4	N.S.

[a] Mean ± S.E.M.
[b] Friedmans two-way analysis of variance by ranks
[c] Monocytes excluded from analysis using 90° light scatter parameters

Results

The preoperative subset values (mean ± SE) for the six patients were almost identical to those observed in age sex matched controls and therefore these normal data are not included. Following surgery the total white cell count increased largely as a result of a significant increase in the numbers of granulocytes (Table 2). In contrast the total lymphocyte numbers decreased significantly reaching a trough at the 4th postoperative day. This was due to a significant decrease of T cells of both major subsets (Table 2).

Some alteration of the relative proportions of the Leu-$3a^+$ and Leu-$2a^+$ subsets resulted in small changes of the Leu-$3a^+/2a^+$ (T "helper/suppressor") rations but these were not statistically significant. Similarly, the total numbers of $HLADR^+$ and HNK-1^+ cells declined following surgery but again, this was not statistically significant (Table 2).

Discussion

In this preliminary study we have shown a pronounced reduction in circulating Th and Ts lymphocyte populations following anaesthesia and surgery for benign disease of the upper urinary tract. On the other hand the Th/Ts ratio (regarded by many as an indicator of immunological status),

and the circulating B cells, NK and K cell levels did not change significantly. Certain of these observations are at variance with those of Miller et al. [3] but this may be a reflection of the different analytical techniques used in the two studies.

At the present time we cannot distinguish between effects due to anaesthesia and surgery. However a number of observations would suggest that the effect is probably cortisol mediated. In the first place T cell levels are influenced by exogenous hydrocortisone [1] and increased plasma cortisol levels are a well documented sequel to surgery performed under general anaesthesia [11]. Furthermore we have shown that the normal circadian variation in peripheral blood T cell subset numbers bears an inverse relationship to endogenous blood cortisol levels [9]. It is highly likely therefore that the observed reduction in T cell subset numbers is a result of increased cortisol secretion resulting in a redistribution of these cells to extravascular compartments. If this is the case then it would also appear that the other lymphocyte subpopulations studied, namely B cells, NK and K cells, are less steroid sensitive than T cells. Further support for this is forthcoming from our previous studies on circadian variations of lymphocyte subpopulations in normal individuals [9].

The consequences, if any, of the marked depression in circulating T cell subset levels following surgery remain to be established. Nevertheless it is highly likely that these observations are relevant to the infection problems which may accompany surgery [5, 6] and to the rapid progression of malignant disease occasionally observed following incidental surgery [11].

Although the number of patients investigated in this study is small we feel these preliminary results emphasise that studies of this type could be extremely relevant to patient care. We conclude that further studies on circulating lymphocyte subset levels in patients undergoing surgery are warranted. If however studies of this type are contemplated it should be emphasised that the study design takes into account the known circadian variation of lymphocyte subsets and the need for standardised blood separation and analytical techniques. Finally we also conclude that the current practice of determining Th/Ts ratios alone should be avoided as it may fail to detect significant changes in the total numbers of the constituent populations.

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Professor
Dr. G. D. Chisholm
Department of Surgery
University of Edinburgh
Teviot Place
Edinburgh EH8 9AG
UK