IMMUNOGLOBULIN-CONTAINING CELLS IN NORMAL AND INFLAMED GENITALIA OF BUFFALO BULLS

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ABSTRACT

An indirect peroxidase-antiperoxidase (PAP) technique was used for identifying specific immunoglobulin-containing cells in normal and inflamed genitalia of buffalo bulls. Rabbit anti bovine immunoglobulins were used as the primary antisera. This study indicated that the distribution of immunoglobulin-containing cells in the genitalia of buffalo bulls was comparable to that in bulls and rams. IgA-containing cells were the most prevalent labelled cells in the penile urethra, whereas in other organs IgG- containing cells were the most common. IgM-containing cells were rarely observed in any tissue. The densities of immunoglobulin-containing cells (number of the cells/high power field) in normal tissues were not significantly different ($P > 0.05$) to those in inflamed tissue studied but differences were very significant within groups of animals ($P < 0.001$). Immunoglobulin-producing cells in the urethral mucosa, due to their superficial position, were considered to make a significant contribution to the humoral immune system of the penis and prepuce.

ABSTRAK

Teknik peroksidase-antiperoksidase (PAP) tak langsung digunakan untuk mengidentifikasi sel-sel yang mengandung imunoglobulin pada alat kelamin kerbau jantan yang normal dan yang meradang. Imunoglobulin kelinci anti sapi (rabbit anti bovine immunoglobulins) digunakan sebagai anti-serum primer. Penelitian ini menunjukkan bahwa distribusi sel-sel yang mengandung imunoglobulin pada alat kelamin kerbau jantan sama dengan yang dilaporkan pada alat kelamin sapi jantan dan domba jantan. Sel-sel yang mengandung imunoglobulin A adalah yang paling banyak jumlahnya pada mukosa uretra, sedangkan pada organ-organ yang lain yang terbanyak jumlahnya adalah sel-sel yang mengandung imunoglobulin G. Sel yang mengandung imunoglobulin M jarang dijumpai pada semua jaringan. Densitas sel yang mengandung imunoglobulin (jumlah sel tersebut/lapangan pandang mikroskop perbesaran objektif 40 x) pada jaringan normal tidak berbeda nyata ($p > 0{,}005$) dengan densitas pada jaringan yang meradang, tetapi perbedaan densitas di dalam kelompok hewan adalah sangat nyata ($p < 0{,}001$). Sel-sel yang mengandung imunoglobulin pada mukosa uretra diduga memainkan peranan yang sangat penting dalam sistem pertahanan lokal, karena sel-sel tersebut letaknya sangat dekat ke permukaan.

INTRODUCTION

The local humoral immune system in male genitalia has been investigated in bulls (Corbeil *et al.*, 1976; Bier *et al.*, 1977; Flower *et al.*, 1982), and in rams (Foster, 1987). In buffalo bulls, however, no such work has been conducted. The aim of the present study therefore was to ascertain the prevalence of immunoglobulin-containing cells in various parts of the genitalia of buffalo bulls that were normal or inflamed.

MATERIALS AND METHODS

Formalin or Bouin's-fixed, paraffin-embedded tissues from normal penis, testis, epididymis, seminal vesicle and ampulla (from three animals), balanoposthitis (eight animals), urethritis (six animals), interstitial orchitis (nine animals), interstitial epididymitis (six animals), seminal vesiculitis (four animals) and ampullitis (four animals) were used for the immunohistological study. Sections from formalin-fixed, paraffin-embedded bovine lymph nodes were used as positive controls.

For identifying specific immunoglobulin-containing cells (Ig-CC), an indirect peroxidase-antiperoxidase (PAP) technique was used. The staining procedures were adopted from Foster (1987), with some modification.

Rabbit anti bovine immunoglobulins (Nordic Immunological Laboratories, Tilburg, the Netherlands), instead of anti buffalo immunoglubulins, were used as the primary antisera. The reason for this was that anti buffalo immunoglobulins were not available commercially and a previous study indicated that the immunoglobulins of both species have a close antigenic homology (Kulkarni *et al.*, 1973).

The immunoglobulin-containing cells were scored by counting the number of the cells in 10 – 20 high power fields (HPF) (40x objective) in single sections of each tissue. The fields were chosen randomly in normal tissues or were located in or around the lesions in inflamed tissue. A repeated measures analysis of variance was performed, after the data were transformed into square root values, using "Statistix II" (an interactive statistical analysis program for microcomputer, Roseville, USA).

RESULTS

The prevalence or density of Ig-CC in various parts and in various conditions of the buffalo genitalia is presented in Table 1.

The densities of the labelled cells were higher in the inflamed tissues than in normal tissues. However, none of the differences was significant. The differences in the number of the labelled cells within animals with the same condition was, however, highly significant ($P < 0.001$). A considerable variation in the cell numbers/HPF was even observed between blocks of tissue taken from different parts of the same penis.

Except in the penile epidermis and urethra, IgG-CC were present in the highest number and IgM-CC were the lowest. These latter cells were present in occasional fields only and their density was never higher than 3 IgM-CC/HPF.

The highest mean numbers of Ig-CC were observed in the penis and urethra. In these organs the mean numbers of IgA-CC/HPF were often higher than that of IgG-CC, especially in the urethra. In the penis the Ig-CC were concentrated mainly in the dermal papillae (Figure 1), whereas in the urethra the Ig-CC were scattered along the urethral mucosae, some of them being very close to the lumen of the urethra (Figure 2). The apical surface of the epithelial cells lining the urethral lumen was intensely stained for IgA. In some cases, however, occasional superficial epithelial cells were deeply stained for IgG (Figure 3).

In the normal testis, epididymis, seminal vesicle and ampulla IgM-CC were never observed and only a few IgG- and IgA-CC were present. In one case of interstitial orchitis, IgG-CC were present in very high number (37 IgG-CC/HPH) but in the eight remaining cases of interstitial orchitis, IgG-CC numbers were low. In the (presumed) parasitic periorchitis cases, the IgG-CC, which were present in relatively high numbers, were located around the necrotic centres.

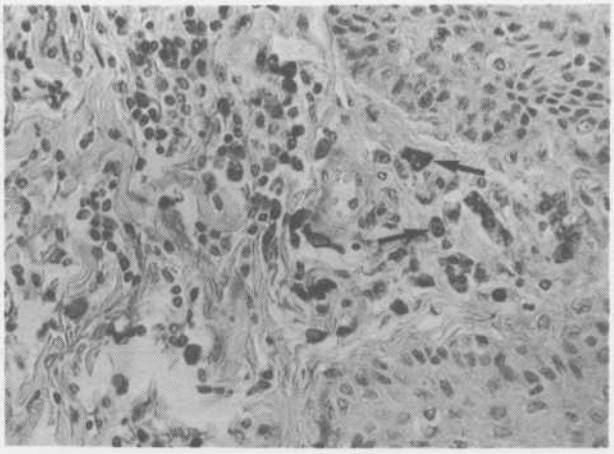

Figure 1. Photomicrograph of normal penis showing darkly stained IgG-containing cells (arrows) in the dermal papillae. PAP, haematoxylin counterstain

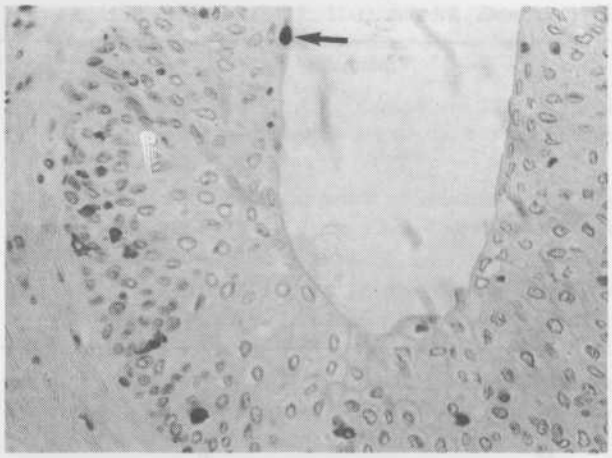

Figure 2. Photomicrograph of normal penile urethra showing heavily stained IgA-containing cells along the urethral mucosa. Some of them (arrow) were located adjacent to the urethral lumen. PAP, haematoxylin counterstain

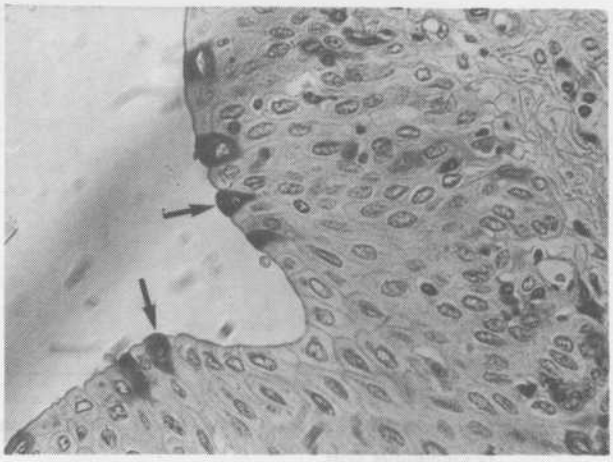

Figure 3. Photomicrograph of normal penile urethra showing heavily stained IgG-containing superficial epithelium (arrows). PAP, haematoxylin counterstain

Table 1. Prevalence of isotope specific immunoglobulin-containing cells (Ig-CC) in the genitalia of normal buffalo bulls and buffalo bulls with inflammation of the genitalia

	Normal tissue		Inflamed tissue	
Ig isotope	Number of animals examined	Number IgCC/HPF* (mean ± S.D.)	Number of animals examined	Number IgCC/HPF* (mean ± S.D.)
	Penis		Balanitis	
G		2.2 ± 3.0		13.2 ± 18.1
A	3	4.6 ± 4.8	8	1.6 ± 2.7
M		0.09 ± 0.4		0
	Urethral mucosa		Urethritis	
G		0.3 ± 0.6		8.1 ± 10.8
A	3	12.2 ± 5.6	6	8.8 ± 9.3
M		0		0.2 ± 0.5
	Testis		Interstitial orchitis	
G		0.4 ± 0.9		6.3 ± 14.8
A	3	0.02 ± 0.1	9	0.2 ± 0.5
M		0		0
	Epididymis		Interstitial epididymitis	
G		0.2 ± 0.5		1.6 ± 3.0
A	3	0	6	0.01 ± 0.01
M		0		0
			Spermatic granuloma	
G			2	1.0 ± 1.7
A				0.05 ± 0.2
M				0
	Seminal vesicle		Seminal vesicultis	
G		0		0.7 ± 2.2
A	3	0	4	0.03 ± 0.2
M		0		0
	Ampulla		Ampullitis	
G		0.1 ± 0.3		0.8 ± 1.8
A	3	0.03 ± 0.2	4	0.2 ± 0.7
M		0		0.07 ± 0.3
	Tunica vaginalis and Tunica albuginea		Presumed parasitic periorchitis	
G				4.6 ± 6.1
A	(not examined in		6	0.2 ± 0.7
M	normal animals)			0.02 ± 0.1
			Secondary lymphoid tissue in the tunica albuginca	
G				0.9 ± 2.0
A			4	0
M				0

* HPF = High Power Field microscopy

Note: No significant differences between normal and inflamed tissues ($p > 0.05$) were observed in regard to prevalence of Ig-containing cells

DISCUSSION

The reason for statistically undetectable differences in the density (number of IgCC/HPF) between normal and inflamed tissues, as shown in this study, was considered to be the high variation in the number of the labelled cells within groups of tissues and pathological conditions. If the sample size in this study had been larger, the differences could have attained significance. High variation in the number of IgCC has also been observed in the prepuce of bulls (Flower *et al.*, 1982), the genitalia of rams (Foster, 1987) and the genitalia of mares (Widders *et al.*, 1985). Such high variation results from the fact that the number of plasma cells is related, in part, to the age of animals (Mosaheb, 1973; Bier *et al.*, 1977; Campero, 1988) and also to the area of the organ from which the tissue blocks are taken. In inflamed tissue, the number of the labelled cells also depends on the duration, severity, and cause of the inflammation.

The fact that a satisfactory result was obtained by using anti bovine immunoglobulin, instead of anti buffalo immunoglobulin, in the present study supported the previous finding (Kulkarni *et al.*, 1973) that a close antigenic homology exists between buffalo and bovine immunoglobulin.

The finding that IgCC in the urethral mucosae were located very close to the lumen indicated that these cells were possibly the main source of the immunoglobulin in the penile urethra and preputial cavity. The significance of the finding, that some epithelial cells lining the urethral lumen were heavily stained for IgG, was unclear. Such staining could, however, be an indication of intracellular diffusion or just artefact. Uptake of protein including all isotypes of immunoglobulin into superficial epithelial cells is common if their membranes are damaged due to degeneration (Brandtzaeg, 1974).

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