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Characterisation of macaque testicular leucocyte populations and T-lymphocyte immunity

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ABSTRACT

The rodent testis is well established as a site of immune privilege where both innate and acquired immune responses are suppressed. Immune cells and responses within human or non-human primate testes, by contrast, are poorly characterised. This study used multi-colour flow cytometry to characterise the leukocytes in testicular cells isolated from 12 young adult pigtail macaques (*Macaca nemestrina*) by collagenase dispersal, and to measure the cytokine responses of macaque testicular T-lymphocytes to mitogens. B-lymphocytes and granulocytes were present in very low numbers (0.24% and 3.3% of leukocytes respectively), indicating minimal blood contamination. A median of 30.8% of the recovered testicular leukocytes were CD3+ lymphocytes, with CD4 and CD8 T-lymphocyte proportions similar to those in the blood. The proportion of naïve T-lymphocytes in the testis was low, with significantly higher frequencies of central memory cells, compared with the blood. A median of 42.7% of the testicular leukocytes were CD163+ macrophages, while 4.5% were $CD14^{+}CD163^{-}$ monocyte-like macrophages. Small populations of myeloid and plasmacytoid dendritic cells, NK cells and NKT cells were also detected. Following mitogen stimulation, 19.7% of blood T-lymphocytes produced IFNγ and/or TNF, whereas significantly fewer (4.4%) of the testicular T-lymphocytes responded to stimulation. Our results characterise the immune cells within the adult macaque testis and identify a suppression of T-lymphocyte responses. This study provides a baseline to examine the immunology of the primate testis and suggests that testicular immune privilege could also be present in primates.

1. Introduction

The rodent testis is well established as a region of immune privilege, where both innate and acquired immune responses are suppressed. This may serve to protect the immunogenic germ cells from acquired immune responses and from the deleterious effects of inflammation (Meinhardt and Hedger, 2011). Currently, there are few studies that have addressed the presence or absence of immune privilege in the testes of humans or non-human primates. Impeding this area of research is the relatively poor characterisation of the leucocyte populations of the primate testis.

The testicular leukocytes reside in the testicular interstitium, and share this compartment with the Leydig cells, connective tissue cells such as fibroblasts and pericytes, and vascular endothelial cells. Leukocytes are never found in the seminiferous tubules under normal conditions (Hedger, 1997). In all species studied to date, the majority of testicular leukocytes are macrophages (Hedger, 2002). The

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macrophage population of the rat testis is the best characterised and consists primarily of resident macrophages that are $CD163^+$. It also includes monocyte-like macrophages that lack CD163, but express other macrophage markers such as CD68 (i.e. $CD68^+CD163^-$) (Wang et al., 1994; Winnall and Hedger, 2013). $CD68^+$ macrophages have been detected in the human and macaque testis (Pöllänen and Niemi, 1987; Frungieri et al., 2002; Shehu-Xhilaga et al., 2007), but the macrophage populations of these species are otherwise poorly characterised. However, data from the human testis suggest that both $CD163^+$ and $CD163^-$ macrophages are present (Frungieri et al., 2002). These testicular macrophages play roles in the response to infection, the regulation of spermatogenesis and steroidogenesis, and are proposed to contribute to immunosuppression in the testis (Hales, 2002; Hedger, 2002; Winnall and Hedger, 2013).

In rat and human testes, T-lymphocytes make up an estimated 10–20% of the leukocytes (El-Demiry et al., 1985; Pöllänen and Niemi, 1987) with $CD8^+$ T-lymphocytes present as the predominant population (Ritchie et al., 1984; Pöllänen and Niemi, 1987; Wang et al., 1994; Tompkins et al., 1998; Jacobo et al., 2009). Smaller populations of dendritic cells, mast cells, eosinophils and natural killer (NK) cells have also been detected in the testes of rodents and some other species (Derrick et al., 1993; Itoh et al., 1995; Anton et al., 1998; Fijak et al., 2005; Fijak and Meinhardt, 2006; Rival et al., 2006), but these remain largely uncharacterised in the primate testis. Neutrophils and B-lymphocytes, which are commonly found in large numbers in the circulation, have not been detected in rodent testes under normal, homeostatic conditions (Hedger, 1997; Tompkins et al., 1998).

The mechanisms behind immune privilege are incompletely defined, but studies in rodents have proposed that anatomical sequestration of germ cells by the Sertoli cell tight junctions, potential immunosuppressive properties of steroid hormones, unique MHC composition inside seminiferous tubules of rodents and humans, as well as the actions of testicular macrophages, contribute to tolerance in the male gonads (Pöllänen and Niemi, 1987; Fiszer et al., 1997; Hutter and Dohr, 1998; Liva and Voskuhl, 2001; Cheng and Mruk, 2002; Hedger and Hales, 2006; Winnall et al., 2011). At least in rodents, the responses of the T-lymphocyte population of the testis to foreign antigens are believed to be inhibited or altered, leading to a prolonged tolerance (Head and Billingham, 1985; Dai et al., 2005; Nasr et al., 2005; Winnall et al., 2011). Local production of immunosuppressive factors such as IL-10, TGFβ, activin A and the presence of immunosuppressive lyso-glycerophosphocholines, which regulate T-lymphocyte activation and survival, are suspected to promote this diminished response of testicular T-lymphocytes (Pollanen et al., 1993; Foulds et al., 2008; Winnall et al., 2011; Bistoni et al., 2012; Hedger and Winnall, 2012). The response of primate testis T-lymphocytes to activation has not been characterised. The present study aims to characterise the leukocytes present in the macaque testis and their response to activation by mitogens.

Table 1
Antibodies.

Antigen[a]	Fluorophore	Clone	Titre	Catalogue number[b]
CD3	Alexa fluor 488	SP34.2	1/200	557705
CD3	Pacific blue	SP34.2	1/100	558124
CD3	APC-Cy7	SP34.2	1/100	557757
CD4	Alexa fluor 700	L200	1/100	560836
CD8	PerCP	SK1	1/70	347314
CD8	APC-H7	SK1	1/100	641400
CD8	PE-Cy7	SK1	1/1000	335787
CD8	APC	SK1	1/800	340584
CD11c	BV711	3.9	1/20	301629[c]
CD14	PE-Cy7	M5E2	1/66	557742
CD14	APC-H7	MPϕ9	1/100	560270
CD16	APC-H7	3G8	1/40	560195
CD19	PE	HIB19	1/20	555413
CD20	PE-Cy7	L27	1/67	335793
CD25	Alexa fluor 488	BC96	1/50	53-0259-42[d]
CD28	PerCP-Cy5.5	L293	1/25	337181
CD45	V450	D058-1283	1/100	561291
CD49d	–	L25	N/A	340976
CD66	PE	B6.2	1/200	551478
CD68	FITC	Y1/82A	1/50	11-0689-41[d]
CD95	FITC	DX2	1/12.5	556640
CD123	APC	7G3	1/125	560087
CD159a (NKG2A)	APC	Z199	1/66	A60797
CD161	BV605	HP-3G10	1/40	339915[c]
CD163	PE	GHI/61	1/25	560933
Lin1	FITC	[e]	1/40	340546
HLA-DR	PerCP-Cy5.5	L243	1/20	552764
Vα7.2	PE	3C10	1/40	351705
TNF	PE-Cy7	mAb11	1/100	557647
IFNγ	APC	B27	1/800	554702

[a] All were raised to human antigens except non-human primate CD45.
[b] All were from BD Biosciences except where indicated.
[c] Purchased from BioLegend.
[d] Purchased from eBioscience.
[e] Lin1 FITC comprises CD3, CD14, CD16, CD19, CD20 and CD56, which are clones SK7, 3G8, SJ325C1, L27, MϕP9, and NCAM16.2, respectively.

2. Materials and methods

2.1. Animals

Testes were sourced from 12 healthy young adult male pigtail macaques (*Macaca nemestrina*) that were involved in an unrelated study. These animals were uninfected controls and no procedures were performed that could have affected the testes. Animals were euthanised using ketamine sedation (1 mg/kg) and pentobarbitone (0.5 ml/kg) and orchidectomy was performed immediately at autopsy. Concurrent blood samples were collected from animals in heparinised collection tubes. Animals were housed in the Australian Animal Health Laboratory and the CSIRO Animal Health animal ethics committees approved all studies.

2.2. Reagents and antibodies

All antibodies and other reagents were purchased from BD Biosciences (San Jose, CA, USA) and raised against human antigens unless otherwise indicated. Antibodies are described in Table 1 and were all mouse monoclonals raised against human antigens, with the exception of