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Stimulation of prostaglandin formation by an antigen- and Ia-restricted T-cell-macrophage interaction

J. Schnyder[1], M. Baggiolini[1] and M. H. Schreier[2]

The [1] Wander Research Institute, a Sandoz research unit, Wander Ltd., Berne, Switzerland; and [2] preclinical Research, Sandoz Ltd., Basel, Switzerland

Abstract

Stimulation of macrophages by zymosan phagocytosis triggers the respiratory burst and induces an early release of lysosomal hydrolases and E-type prostaglandins (PGE). We have studied whether antigen presentation by macrophages to helper T-cells elicits a comparable sequence of events. Cloned T-helper cells specific for hen egg albumin (EA) were added to histocompatible or histoincompatible resident mouse peritoneal macrophages in the presence of EA or an unrelated antigen, and the changes in biochemical parameters were monitored. The interaction between macrophages, T-helper cells and EA induced the production of PGE, but no release of lysosomal hydrolases or activation of the respiratory burst. In addition T-cell proliferation was observed. By contrast, no proliferation and no biochemical changes were observed when histoincompatible macrophages or unrelated antigen were used. When the experiments were done in the presence of indomethacin to inhibit PGE release, T-cell proliferation was enhanced. These results suggest that the PGE released may exert a feed-back control of the T-cell response.

Introduction

It has been shown that clones of T-helper cells which are responsive to a given antigen proliferate following exposure to the antigen and histocompatible phagocytes [1, 2]. We have now used a similar experimental set-up to study the biochemical responses of the phagocytes following antigen presentation. The release of E-type prostaglandins (PGE) [3], H_2O_2 and lysosomal hydrolases [4], and proliferation of the T-helper cells [2] were tested.

[1] present address: Theodor Kocher Institute, CH-3009 Berne, Switzerland

Correspondence to: Dr. J. Schnyder, Research Institute, Wander Ltd., P.O. Box 2747, CH-3001 Berne, Switzerland.

Results

Biochemical responses of macrophages interacting with T-cells

In a first set of experiments, histocompatible C57/Bl peritoneal macrophages and antigen-primed, cloned T-helper cells were cultured in the presence of either the priming or an unrelated antigen, and the release of PGE, β-glucuronidase and H_2O_2 was measured. The T-cells were either present for the first 3 h or for the whole period of 24 h. Macrophages released high amounts of PGE when cultured with the histocompatible, hen egg albumin-dependent T-helper cells (T'_{EA}) and hen egg albumin (EA) as the priming antigen. Under the same conditions, but in the presence of the unrelated antigen, pigeon egg albumin, PGE

Table 1
Production of E-type prostaglandin by resident macrophages in response to zymosan and to T-helper cells and antigens.

Mouse strain	Additions	Prostaglandin production (ng per well)+		
		A	B	C
C57/Bl (histocompatible)	none	1.6±0.23 (9)	3.2±0.28 (9)	3.3±0.97 (6)
	zymosan	4.1±0.16 (6)*	13.8±0.87 (9)*	3.5±0.40 (2)
	T_{EA}+EA	1.7±0.16 (6)	8.1±1.68 (9)*	7.8±0.98 (6)*
	T_{EA}+PIA	1.2±0.13 (6)	2.4±0.30 (9)	3.0±0.63 (6)
	EA	1.8±0.06 (3)	1.6±0.06 (3)	1.0±0.06 (3)
B 10.A (4R) (histoincompatible)	none	1.6±0.08 (3)	3.9±0.56 (3)	0.7±0.07 (3)
	zymosan	6.7±0.15 (3)*	16.3±0.08 (3)*	3.6±0.36 (3)
	T_{EA}+EA	0.8±0.07 (3)*	2.4±0.31 (3)*	1.5±0.13 (3)*
	T_{EA}+PIA	0.9±0.15 (3)*	2.1±0.11 (3)*	1.3±0.04 (3)*
	EA	1.6±0.06 (3)	0.0±0.01 (3)*	0.7±0.16 (3)

+ Means and SEM from experiments run in triplicate (number of determinations are given in parenthesis).
* $p < 0.01$ in all other cases $p > 0.05$.
Each 5×10^6 resident macrophages and T-helper cells in 1 ml medium 199 containing 1% acid-treated FCS (4) were cultured on 24-well plates in the presence or absence of stimuli (zymosan, 8 particles/cell; 100 µg/ml hen egg albumin (EA) or pigeon egg albumin (PIA)) and the amount of PGE produced in different time intervals was measured.
A: Stimuli present for 1 to 3 h. Prostaglandin production in that 1- to 3-h period.
B: Stimuli present for 24 h and prostaglandin production during 24-h period.
C: Stimuli present for 3 h, after that time the medium was replaced and the cells were cultured for 21 h in the absence of stimuli. The production of PGE after the stimulation period, i.e. t_3 to t_{24}.

Table 2
Effect of prostaglandin synthesis inhibition on T-helper cell proliferation.

Source of macrophages	Additions		Experiment					
	T_{EA}+EA	Indo-methacin (µM)	1		2		3	
			pg+	cpm+	pg+	cpm+	pg+	cpm+
C57/Bl	–	–	682	127	595	51	–	43
	+	–	1045	4363	1099	579	–	4726
	+	0.01	196	12850	142	1770	–	7416
	+	0.1	52	19019	0	3934	–	14704
	+	1.0	39	21911	0	5358	–	16595
B 10.A (4R)	–	–	770	64	482	71	–	41
	+	–	661	136	501	60	–	53
	+	0.01	180	144	75	54	–	73
	+	0.1	50	194	0	70	–	121
	+	1.0	42	137	0	48	–	45
B 10.A (5R)	–	–	602	102	570	48	–	61
	+	–	1570	5784	860	1074	–	3342
	+	0.01	271	12320	132	2468	–	6055
	+	0.1	79	22378	0	6817	–	11304
	+	1.0	64	29174	8	7634	–	16987
MBR	–	–	647	177	267	32	–	29
	+	–	679	145	368	44	–	42
	+	0.01	163	158	58	191	–	91
	+	0.1	63	105	0	118	–	93
	+	1.0	56	85	0	83	–	80

+ Mean values from duplicate cultures. Data from three independent experiments are given. Each 4×10^5 resident macrophages and T-helper cells per well were cultured on 96-well plates in 0.2 ml of the medium C (2) containing 5% of acid-treated fetal bovine serum instead of serum substitute. After 24 h, 100 µl of medium were removed for PGE assay and ^{3}H-thymidine was added. The incorporated radioactivity was measured 48 h later.

production remained at control levels. Furthermore, the macrophage response was genetically restricted. Enhanced PGE formation was observed in cultures of histocompatible cells (C57/Bl; B10.A[5R]), but not in cultures of histoincompatible cells (B10.A[4R] or MBR) (Table 1 and 2). Phagocytosis of unopsonised zymosan particles induced the expected burst of PGE (Table 1), H_2O_2 and β-glucuronidase release (not shown). The two latter changes were not observed in the presence of T-helper cells and antigen, indicating that on interplay with T-helper cells macrophages do not secrete lysosomal hydrolases or mount a respiratory burst.

Effect of prostaglandin synthesis inhibition on T-cell proliferation

Since PGE release was found to be a selective response of macrophages cooperating with T-helper cells, it was of interest to test the effect of a cyclooxygenase inhibitor on the EA-induced response of the T-cell clones (T'_{EA}). Proliferation was only observed in the presence of histocompatible macrophages. Addition of EA enhanced thymidine incorporation 10- to 100-fold over control values. In the presence of 0.01–1 μM indomethacin a further increase by at least 3-fold was observed (Table 2).

Discussion

Unlike zymosan phagocytosis which triggers a general biochemical activation of macrophages as measured by an early release of lysosomal hydrolases and PGE, and a delayed secretion of plasminogen activator, the interaction between histocompatible macrophages, specific T-helper cells and the priming antigen only induced the production of PGE. This cell hormone is known to inhibit lymphocyte functions, including Ia expression and lymphokine production. Its selective release by macrophages mediating the response of T-cells to antigen and the effect of PGE release inhibition by indomethacin as shown by our study indicate that PGE may fulfill an immunoregulatory function in addition to the induction of inflammatory reaction observed following T-cell clones and antigen injection to mice paw [5].

References

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