STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	Title: "An in-vitro study"; Abstract: "co-culture system ... divided into three groups: control, model and inhibition"
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1	Abstract reports: PBMCs/FLS from 40 RA patients and 40 controls; 48h co-culture; assessed viability, apoptosis, cytokines (TNF-α, IL-1β), COX-2, Bax, Bcl-2, TNFR mRNA, CD28/CD80 expression; results show model vs control differences and PDTC effects; conclusion stated
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	1-2	Covers: RA as autoimmune disease with T cell dysfunction; B7/CD28 as key costimulatory signals; TNF/TNFR family in immune regulation and NF-κB pathway; existing knowledge gaps on their interplay in RA
Objectives	3	State specific objectives, including any prespecified hypotheses	1	Hypothesis: TNF/TNFR may regulate B7/CD28 expression via NF-κB in RA, affecting T cell activation and synovial cell apoptosis; objective: observe changes in these molecules after co-culture and NF-κB blockade
Methods				
Study design	4	Present key elements of study design early in the paper	2	Cross-sectional in-vitro comparative study; RA patients and healthy controls consecutively recruited; PBMCs and FLS co-culture system with control, model, inhibition (PDTC) and blockade (CTLA-4

				Ig) groups
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	2	Sichuan Orthopedic Hospital; RA synovial tissues from knee replacement/synovectomy; control tissues from trauma amputation/arthroscopy; healthy controls from Medical Examination Center; 48h co-culture; submission/revision dates indicate study period
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	2	RA group: 40 patients (13M/27F, age 26-55), consecutively recruited, ACR/EULAR 2010 criteria, DAS28-ESR>3.2; exclusions: other autoimmune diseases, biologics/JAK inhibitors within 3 months, high-dose glucocorticoids within 1 month, malignancy, severe organ dysfunction. Controls: 40 volunteers (15M/25F, age 26-54), age/sex matched, no arthritis/autoimmune disease, no infection/medication within 3 months
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	2	Age and sex matched; RA group: 13M/27F, 26-55y; Control: 15M/25F, 26-54y; 1:1 ratio
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	2-4	Outcomes: cell viability (MTT), apoptosis (Annexin V/PI), cytokines TNF-α/IL-1β (ELISA), COX-2/Bax/Bcl-2 (Western blot), TNFR mRNA (RT-PCR), CD28/CD80 on $CD4^+$ /$CD8^+$ T cells (flow cytometry). Exposure: RA PBMCs. Intervention: PDTC (100μM), CTLA-4 Ig

				(100mg/mL). RA diagnosis: 2010 ACR/EULAR criteria
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	2-4	Dedicated subsections for each assay: MTT (96-well plates, 490nm), Annexin V-FITC/PI flow cytometry, surface marker staining with specific antibody clones (CD4 PerCP-Cy5.5, CD8 APC, CD28 PE, CD80 FITC), ELISA (R&D Systems kits), Western blot (primary antibodies: Bax, Bcl-2, COX-2, GAPDH), RT-PCR (SYBR Green, 2^-ΔΔCt method with GAPDH). All methods applied identically across groups
Bias	9	Describe any efforts to address potential sources of bias	2-4	FLS purity >95% confirmed by flow cytometry ($CD90^+$ /$CD14^-$ /$CD45^-$); isotype controls and FMO controls for flow cytometry; five replicate wells per group; experiments independently repeated three times
Study size	10	Explain how the study size was arrived at	2	40 RA patients and 40 controls enrolled; based on preliminary experiments and comparable published studies

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Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why		Expressed as mean ± SD
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4	One-way ANOVA; LSD-t test if homogeneity of variance met, Welch's test + Games-Howell if not; P<0.05 considered significant
		(*b*) Describe any methods used to examine subgroups and interactions	Not applicable	No subgroup analyses, no missing data, no sampling strategy adjustments
		(*c*) Explain how missing data were addressed	Not applicable	no sampling strategy adjustments
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	Not applicable	Not applicable
		(*e*) Describe any sensitivity analyses	Not applicable	no sensitivity analyses
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	2	40 RA patients and 40 controls enrolled; all samples successfully processed; n=5 per experimental group for all assays
		(b) Give reasons for non-participation at each stage	Not applicable	No non-participation or loss to follow-up
		(c) Consider use of a flow diagram	Not applicable	no flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	2	Age and sex provided for both groups; RA group: 13M/27F, 26-55y; Control: 15M/25F, 26-54y
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable	No missing data
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)		
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	5-7	Tables 2-5 and Figure 1 report all outcomes with N=5, mean ± SD, F values, and P values for all groups

		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*2-7*	All outcome measures were summarized as mean ± standard deviation (SD) for each experimental group (N=5 per group) in the Results section. Cell viability at 24, 48 and 72 hours was reported as OD values (Table 2); total apoptosis rate (early + late) was presented as percentages (Fig. 1); apoptosis-related proteins Bcl-2 and Bax were shown as relative protein expression levels (Table 3); inflammatory cytokine concentrations (TNF-α, IL-1β) and COX-2 protein expression, as well as TNF-R1/R2 mRNA relative expression ($2^{-\Delta\Delta Ct}$ method), were reported in Table 4; and the positive expression rates of CD28 and CD80 on $CD4^+$ and $CD8^+$ T cells were presented in Table 5. No missing data occurred for any of the outcome variables.
		Cross-sectional study—Report numbers of outcome events or summary measures	2	The exposure of interest was RA patient-derived PBMCs. The control group received PBMCs from healthy donors (unexposed, N=5), while the model and inhibition groups received RA patient-derived PBMCs (exposed, N=5 per group). All exposure assignments were clearly defined and consistently applied across experimental replicates.
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	2-7	All pairwise comparisons reported with exact P values;

				e.g., model vs control: P<0.001 for most outcomes; inhibition vs model: P<0.001 for IL-1β, COX-2, apoptosis, CD28/CD80 on $CD4^+$, but P=0.172 for TNF-α and P=0.052/0.791 for TNFR mRNA
		(b) Report category boundaries when continuous variables were categorized	Not applicable	No categorized continuous variables; no relative risk estimates
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable	No categorized continuous variables; no relative risk estimates

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses		Not applicable
Discussion				
Key results	18	Summarise key results with reference to study objectives	8	NF-κB inhibition reduced FLS viability, increased apoptosis, downregulated Bcl-2, upregulated Bax; decreased IL-1β and COX-2 but not TNF-α/TNFR; restored CD28/CD80 on $CD4^+$ T cells; CD28 blockade attenuated PDTC-induced apoptosis
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8	Small sample size; PDTC has antioxidant/chelating properties affecting other pathways; lack of TNFR-targeted intervention; no functional T cell assays; PBMC-FLS co-culture cannot fully replicate in-vivo microenvironment
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8	Interpretations framed cautiously ("suggests," "may be associated," "insufficient to determine," "remains unclear"); conclusion states NF-κB pathway plays important role but TNF/TNFR relationship remains unclear
Generalisability	21	Discuss the generalisability (external validity) of the study results	8	Small sample size limits broad applicability; findings require validation in animal models (e.g., CIA)
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9	Funding: None

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.