Ischaemic postconditioning protects against reperfusion injury via the SAFE pathway

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1. Introduction

Following acute coronary occlusion, early reperfusion is the current optimal way to rescue the heart. Although essential, reperfusion is associated with cellular damage by activation of deleterious signalling cascades, as early as in the first seconds of reperfusion.1 Attempts in the management of reperfusion injury have recently entered the clinical arena.2–4 Ischaemic postconditioning (IPostC), whereby brief episodes of ischaemia/reperfusion are applied at the onset of reperfusion, is a powerful tool that activates intrinsic prosurvival signalling cascades to limit reperfusion injury.5 IPostC is still often thought to protect via activation of prosurvival kinases Akt and Erk1/2 at the time of reperfusion.6–8 However, recent studies demonstrated that protection with IPostC can occur independently of the activation of the RISK pathway, therefore confirming the existence of multiple protective pathways.9–12

Aims Ischaemic postconditioning (IPostC) is a powerful protective phenomenon that activates prosurvival intrinsic signalling cascades to limit reperfusion injury. We propose that IPostC confers its infarct-sparing effect via activation of the newly described prosurvival Survivor Activating Factor Enhancement (SAFE) pathway, which involves the activation of the cytokine tumour necrosis factor alpha (TNFα) and signal transducer and activator of transcription-3 (STAT-3).

Methods and results Isolated ischaemic/reperfused hearts from TNF knockout, TNF receptor-1 knockout, TNF receptor-2 knockout, cardiomyocyte-specific STAT-3-deficient mice or their respective wildtype, (TNF-WT) or (STAT-3-WT), were postconditioned by ischaemic episodes (IPostC) or with exogenous TNFα 0.5 µg/L (TNF-PostC) at the onset of reperfusion. IPostC reduced infarct size (IS) in TNF-WT and TNFR1−/− hearts (by 33 and 27%, respectively, P < 0.05), whereas hearts from TNF−/− or TNFR2−/− failed to be postconditioned. TNF-PostC reduced IS by 37% (P < 0.05) in STAT-3-WT hearts but failed to protect cardiac-specific STAT-3−/− hearts. Administration of wortmannin, an inhibitor of PI-3 kinase/Akt, or PD98059, an inhibitor of extracellular regulated kinase 1/2 (Erk1/2), during the postconditioning stimulus did not abolish the infarct-sparing effect of TNF-PostC. AG490, an inhibitor of STAT-3, abrogated the protective effect of TNFα. Western blot analysis did not demonstrate the involvement of Akt or Erk1/2 in TNF-PostC, whereas STAT-3 phosphorylation was increased in both IPostC and TNF-PostC.

Conclusion The protective effect of the SAFE pathway is shown in IPostC, with the activation of TNFα, its receptor type 2, and STAT-3. This signalling cascade is activated independently of the well-known Reperfusion Injury Salvage Kinases (RISK) pathway, which involves the kinases Akt and Erk1/2.

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Therefore, our aim was to explore whether TNFα-signalling, through the activation of its receptor-2 (TNFR2), was involved in the protective effect of IPostC and whether this signalling mechanism activated the SAFE pathway rather than the RISK pathway, to confer protection. We used an isolated heart model from wild-type (WT) mice and three different transgenic mice from the same background, TNF receptor-1 knockout (TNFR1−/−), TNF receptor-1 knockdown (TNFR1−/−/−), and a TNF receptor-2 knockdown (TNFR2−/−/−) to demonstrate that binding of TNFα to its receptor-2 during IPostC is indeed required for the protection. Furthermore, pharmacological protein kinase inhibitors and mice which are deficient in the transcription factor, STAT-3 in their cardiomyocytes were used to demonstrate that in the context of IPostC, TNFα confers its cardioprotective effect via the activation of the transcription factor STAT-3 rather than the protein kinases Akt and Erk1/2.

2. Methods

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health [NIH Publication No. 85 (23), revised 1996]. All procedures were approved by the Faculty of Health Sciences Animal Ethics Committee, University of Cape Town. Homozygous TNF-deficient mice (TNF−/−), TNF receptor-1-deficient mice (TNFR1−/−), or TNF receptor-2-deficient mice (TNFR2−/−) and their respective WT, Black6 × 129S (TNF-WT) were used. All these mice were a generous gift from Dr Muazzam Jacobs and Professor Bernhard Ryffel, Department of Immunology, Faculty of Health Sciences, University of Cape Town. Cardiomyocyte-specific STAT-3-deficient mice (STAT-3−/−) from C57Black6 background were created in our facilities by crossing homozygous floxed STAT-3 mice with heterozygous MLC2V-driven Cre recombinase mice, as previously described. All mice were 12–14 weeks of age (weighing 25–30 g).

2.1 Perfusion of isolated mouse hearts

Hearts from adult male TNF−/−, TNFR1−/−/−, TNFR2−/−, cardiomyocyte-specific STAT-3−/− mice and their respective WTs were excised rapidly and perfused retrogradely using the Langendorff apparatus. Isolated hearts were subjected to a 20 min (min) stabilization period followed by 35 min global ischaemia and a 45 min reperfusion period as previously described (see Supplementary material online, Figure S1). At the onset of reperfusion, IPostC was initiated by six alternating cycles of 10 s reperfusion, 10 s ischaemia. In a separate group, pharmacological postconditioning with TNFα was initiated by six alternating cycles of 10 s reperfusion with 0.5 μg/L TNFα (TNF-PostC) (see Supplementary material online, Figure S2). To determine whether STAT-3, Akt, or Erk1/2 were involved, AG490, an inhibitor of the JAK/STAT pathway (100 nmol/L), wortmannin (wort), an inhibitor of the phosphotydinositol-3 kinase (PI-3 kinase)/Akt pathway (100 nmol/L), or PD98059 (PD), an inhibitor of the Mek/Erk1/2 pathway (100 nmol/L) were given during the first 15 min of reperfusion. To confirm the role of the TNF receptors, specific antibodies to TNFR1 or TNFR2 (3 mg/L) were given to TNF-WT mice during the IPostC protocol. The concentration of the antibodies corresponded to the ND50 of the antibodies, as given by the manufacturer.

A minimum of six hearts were used in all groups. Infarct size (IS) was evaluated by triphenyltetrazolium chloride staining and assessed using computerized planimetry (Planimetry+, Boreal Software, Norway) as described previously.

2.2 Western blot analysis

Control or postconditioned mouse hearts were subjected to a 20 min stabilization period followed by 35 min of global ischaemia and 15 min of reperfusion. The hearts were then snap-frozen in liquid nitrogen before being stored at −80°C until protein extraction was performed. Nuclear and cytosolic proteins were extracted from the hearts by homogenization of the tissue in a lysis buffer, as previously described. Phosphorylated states of Akt, Erk1/2, STAT-3 (phospho-STAT-3, tyr705), glycogen synthase kinase-3 beta (phospho-GSK-3β, ser 9) as well as total levels of Akt, Erk1/2, STAT-3, GSK-3β, and glyceraldehyde-3 phosphate dehydrogenase (GAPDH) were analysed by sodium dodecyl sulfate–polyacrylamide gel immunoelectrophoresis (SDS–PAGE) using antibodies from Cell Signaling Technologies. Equal loading was verified with GAPDH. Levels of phosphorylated proteins were normalized to their total protein levels done in the same samples and in the same conditions but on a separate membrane. Relative densitometry was determined with the use of computerized software package (UVI Soft, UVI Band, UVI Tech, Cambridge, UK). A minimum of six hearts was used per group.

2.3 Chemicals and pharmacological agents

Unless otherwise stated, all chemicals were obtained from Sigma-Aldrich Chemicals, Germany. TNFα was obtained from Peprotech Inc., Israel, TNF receptor monoclonal antibodies were obtained from R&D Systems, USA and western blot antibodies from Cell Signaling Technologies, USA.

2.4 Statistical analysis

Data are presented as mean ± SEM. Comparisons between multiple groups were performed by one-way ANOVA followed by Tukey post hoc test (Graph Pad Instat). P < 0.05 was considered statistically significant.

3. Results

3.1 TNFα is required in IPostC

To determine whether TNFα is required in IPostC-mediated cardioprotection, we compared IS (calculated as a percentage of total ventricular volume) in TNF-WT vs. TNF−/− mice following the IPostC protocol of 6 × 10 s cycles of alternating reperfusion and ischaemia (see Supplementary material online, Figure S1). IPostC in TNF-WT mice reduced IS from 52 ± 3% in the ischaemic control group (I/R) to 18 ± 2% for IPostC (P < 0.05; Figure 1). IPostC with

![Figure 1](image-url)
6 × 10 s cycles in the TNF−/− hearts failed to reduce the IS (37 ± 3%, P = ns vs. TNF−/− I/R group, Figure 1).

3.2 TNFα can mimic IPostC in TNF-WT but not TNF−/− mice

Pharmacological postconditioning at the onset of reperfusion with 6× alternating cycles of 10 s reperfusion without TNFα and 10 s reperfusion with 0.5 μg/L TNFα (see Supplementary material online, Figure S2) significantly reduced the IS in TNF-WT mice compared with the ischaemic control group (18 ± 1% for TNF-PostC vs. 52 ± 3% in I/R, P < 0.05; Figure 1). In contrast, the TNF-PostC protocol failed to reduce the IS in TNF−/− mice (55 ± 2%, P = ns vs. I/R in TNF−/− mice, Figure 1).

3.3 TNFR1−/− but not TNFR2−/− mice can be postconditioned

To investigate the role of the TNF receptors in IPostC, we measured IS in TNFR1−/− and TNFR2−/− mice subjected to both IPostC and TNF-PostC (Figure 2A). Ischaemic control hearts from TNFR1−/− and TNFR2−/− mice had an IS of 42 ± 3% and 42 ± 2%, respectively, which was similar to data obtained in TNF-WT mice. IPostC protocol significantly reduced the IS in TNFR1−/− mice (15 ± 1%, n = 6; P < 0.05 vs. TNFR1−/− ischaemia-reperfusion control) but failed to protect the heart in TNFR2−/− mice (38 ± 3%, P = ns, vs. TNFR2−/− I/R control group). Similarly, TNF-PostC decreased the IS to 22 ± 2% in TNFR1−/− hearts, (P < 0.05 vs. its TNFR1−/− I/R control group) but failed to protect the TNFR2−/− hearts (P = ns, vs. TNFR2−/− I/R control group).

We confirmed these results by using specific neutralizing antibodies to each receptor during the IPostC protocol in TNF-WT mice (Figure 2B). Ischaemia-reperfusion-control hearts had an IS of 49 ± 2%. Perfusion of the TNFR1 antibody during the IPostC protocol did not affect the protective effect of postconditioning (19 ± 2%, P < 0.05 vs. ischaemic-reperfusion control group). In contrast, the protective effect of IPostC was lost in the presence of TNFR2 antibody (53 ± 1%, P = ns, vs. ischaemic-reperfusion control group).

3.4 Inhibition of STAT-3 but not Akt or Erk abrogates the protective effect of pharmacological postconditioning with TNFα

To further investigate the mechanism responsible for the cardioprotective effect of TNFα, we measured IS in hearts perfused with an inhibitor of the JAK/STAT pathway (AG490), an inhibitor of the PI-3 kinase/Akt pathway (wort) or an inhibitor of Mek/Erk1/2 (PD98059), during the first 15 min of reperfusion in IPostC or TPostC hearts (Figure 3). The inhibitors did not affect the IS in the ischaemic control group. Addition of wort or PD98059 did not alter the protective effect of TNF-PostC (19 ± 2% and 20 ± 1%, respectively, P < 0.05, vs. 49 ± 2% in the ischaemia-reperfusion control group), whereas PD98059 partially reduced the protective effect of IPostC (29 ± 2%, P < 0.05 vs. I/R + PD98059) and wort completely abrogated the effect of IPostC (43 ± 1%, P = ns vs. 44 ± 0.5% for I/R + W). Inhibition of the JAK/STAT pathway with AG490 during the IPostC or TNF-PostC protocols, abolished the infarct-sparing effect of both postconditioning protocols (47 ± 2% for IPostC; P = ns vs. 47 ± 3% for I/R and 51 ± 2% for TPostC, P = ns, vs. 47 ± 3% for ischaemia-reperfusion control group).

3.5 TNFα failed to mimic IPostC in STAT-3−/− mice

The IS in STAT-3-WT mice compared with the ischaemic control group was significantly decreased by pharmacological postconditioning with 6× alternating cycles of 10 s reperfusion without TNFα and 10 s reperfusion with 0.5 μg/L TNFα initiated at the onset of reperfusion (20 ± 1% for TNF-PostC vs. 52 ± 2% in I/R, P < 0.05; Figure 4). In contrast, the STAT-3−/− mice could not be postconditioned with TNFα (44 ± 3% in I/R vs. 47 ± 1% in TNF-PostC, P = ns; Figure 4).

3.6 Levels of STAT-3, Akt, Erk1/2, and GSK-3 in WT and TNF−/− mice

We examined the levels of STAT-3, Akt, Erk1/2, and GSK-3β during the first 15 min of reperfusion after either IPostC or
TNF-PostC. An increase in phosphorylated STAT-3 [in arbitrary units (AU) from 18 ± 2 for I/R to 54 ± 6, P < 0.05], GSK-3β (from 5 ± 0.4 for I/R to 15 ± 1, P < 0.05), and Erk1/2 (from 9 ± 1 for I/R to 26 ± 2) occurred in the nuclear fraction after TNF-PostC in TNF-WT hearts (Figures 5 and 6 and see Supplementary material online, Figures S3 and S4). In contrast, Akt phosphorylation was decreased after TNF-PostC from 18 ± 2 for I/R to 6 ± 1, P < 0.05. In TNF-WT hearts subjected to IPostC, an increase in phosphorylation of STAT-3 (from 18 ± 2 for I/R to 46 ± 5, P < 0.05) and GSK-3β (from 5 ± 0.4 for I/R to 32 ± 2, P < 0.05) was observed but the Akt level was unchanged. In TNF−/− mice subjected to IPostC, a modest increase in phosphorylated STAT-3 occurred compared with the I/R group, but no increase in GSK-3β was observed in the nuclear fraction after the postconditioning stimulus compared with the I/R group (Figures 5 and 6 and see Supplementary material online, Figures S3 and S4). Of note, phosphorylation of both Akt and Erk1/2 was significantly increased in the nuclear fraction by IPostC compared with I/R in TNF−/− mice.

In the cytosolic fraction, I/R increased phosphorylation of STAT-3, decreased phosphorylation of Erk1/2, and Akt in WT animals (see Supplementary material online, Figures S5–S8). IPostC reduced phosphorylated STAT-3 but increased the phosphorylation of both Akt and Erk1/2 in the cytosol compared with the I/R group. Pharmacological postconditioning with TNFα increased phosphorylation of Erk1/2, whereas phosphorylation levels of STAT-3 remained unchanged in the cytosol.

In the TNF−/− mice, we were surprised to observe an increase of phosphorylation in both STAT-3 and Akt in the cytosol associated with a decrease in phosphorylated Erk1/2 following an IPostC stimulus.

4. Discussion

Our data show, for the first time and possibly counter intuitively, that endogenous or exogenous TNFα, at the time of reperfusion, initiates a novel and RISK-free protective pathway in IPostC. In brief, six episodes of IPostC, performed by alternating cycles of reperfusion and ischaemia in TNF-deficient mice, failed to protect against ischaemia–reperfusion damage. The activation of TNF receptor-2 is implicated in the postconditioning protective effect as TNFR1−/− mice, but not TNFR2−/− mice, could be postconditioned. The protection of IPostC was reduced in the presence of wort, PD98059, and AG490, whereas postconditioning with TNFα could only be abrogated by AG490. Furthermore, the STAT-3−/− hearts could not be postconditioned. Our data demonstrate that protection with IPostC can be achieved via the activation of TNFα and STAT-3, key components of the prosurvival SAFE pathway.

4.1 Cardioprotective role of TNFα

Previously, we have consistently demonstrated, in both in vitro and in vivo experiments, that exogenous TNFα could confer a cardioprotective effect against reperfusion injury in a time and concentration-dependent manner, as reviewed.16 Our group has shown that tissue levels of TNFα in the in vivo rat heart were significantly increased in the area at risk and that various doses of exogenous TNFα administered prior to the ischaemic protocol could be used as a preconditioning stimulus.29 Furthermore, a recent in vivo rat study demonstrated that the protection with IPostC is concomitant with decreased plasma levels of TNFα at reperfusion.7 Our present data demonstrate that TNFα, given immediately at the onset of reperfusion, confers protection in the isolated heart. This is in apparent contradiction to previous reports from isolated rat hearts, suggesting that the release of TNFα, as part of the inflammatory response during the reperfusion phase, contributes to the development of myocardial infarction, contractile dysfunction, and apoptosis.18,30 However, the recent demonstration of a biphasic kinetic pattern of TNFα release in a murine reperfusion model suggests that protection is conferred by the limited release of TNFα during the first few minutes of reperfusion (2–10 min).31 This limited amount of TNFα is then washed out and replaced with a more robust release of TNFα in the later stages of reperfusion. This subsequent increase in TNFα levels is thought to be responsible for its detrimental effects.32 In our present study, we show, for the first time, in an isolated heart model, that during either IPostC or TNF-PostC, the binding of TNFα to TNFR2 at the early stage of reperfusion is crucial for protection. Therefore, a possible explanation of our potentially controversial findings is that the synergetic limited early release/addition of TNFα, together with its binding to TNFR2, at the onset of reperfusion, may limit further harmful production of TNFα during the later stages of reperfusion.

Using TNF−/− mice in our experiments, we were surprised to observe that TNFα postconditioning in these mice failed to protect the heart. In a previous study, our research group demonstrated that the administration of TNFα to TNF knockout mice, 24 h prior to experimentation rescued the protective effect of ischaemic preconditioning in these mice.27 These data suggest that, in TNF knockout mice, the signalling pathways downstream of TNFα may be downregulated. Similar data were obtained in our postconditioning setting in the TNF−/− mice. In these mice, the protective effect of TNF-PostC could be restored following administration of 0.5 ng TNFα, i.p. 24 h prior to the experiments (data not shown). Further functional studies are necessary to evaluate the TNF receptor densities and receptor–ligand interaction to fully explain this ability to rescue the protective effect in TNF−/− hearts.
4.2 TNFα initiates the activation of the prosurvival SAFE pathway rather than the RISK pathway

An established protective pathway, known as the RISK pathway and involving the activation of protein kinases Akt and Erk1/2 in early reperfusion, has been described in both pre- and postconditioning.6,8

A recent study done in pig hearts reported an activation of the kinases involved in the RISK pathway following an IPostC stimulus.9 However, the inhibition of these kinases did not alter the protective effect, therefore questioning the essential role of the RISK kinases in their model.9 Similarly, our present study, performed on a mouse model, demonstrates that pharmacological postconditioning with TNFα is independent of the RISK pathway, as neither PD98059 nor wort abolished the protective effect of TNF-PostC. In contrast, the protective effect of both IPostC and pharmacological postconditioning with TNFα was abolished in the presence of AG490, the JAK/STAT pathway inhibitor. In addition, neither ischaemic nor pharmacological postconditioning with TNFα could protect in STAT-3-deficient mice.

Our new data show the existence of an alternative protective pathway during the reperfusion period of IPostC. This pathway, previously described in ischaemic preconditioning and involving the activation of both TNFα and STAT-3, is now recognized as the SAFE pathway.16,17 Our present data

Figure 5  Phosphorylation of STAT-, Akt, and Erk1/2 in TNF-WT and TNF−/− mice. Bar graphs of data derived from western blots of the nuclear fraction demonstrating phosphorylation of STAT-3 and Akt and Erk1/2 after 15 min of reperfusion in isolated murine hearts; *P < 0.05 vs. I/R. All groups corrected to GAPDH densities and expressed in AU. Abbreviations as described in the text.
strongly support the hypothesis that the recent studies describing a RISK-independent protective effect of IPostC may in fact activate the SAFE pathway.\textsuperscript{9–11}

Our current and past studies suggest that inhibiting either the SAFE or RISK pathway is enough to abolish the infarct-sparing effect of ischaemic pre- or postconditioning.\textsuperscript{14,15,32} Attempts to delineate a possible crosstalk between the two pathways reveal a complex interaction. Hence, in the presence of wort during the ischaemic preconditioning stimulus phosphorylation of STAT-3 was inhibited.\textsuperscript{15} Similarly, Akt failed to be phosphorylated in STAT-3 knockout mice subjected to ischaemic preconditioning.\textsuperscript{15} In contrast, a substantial increase of phosphorylated Akt is observed in TNF\textsuperscript{−/−} mice subjected to IPostC (present data). Further studies are needed to characterize the crosstalk between the SAFE and RISK pathways in both ischaemic pre- and postconditioning.

4.3 Possible downstream targets of SAFE pathway

Inactivation of GSK-3β is a well-established downstream target of the RISK pathway. Our present work aimed to delineate whether GSK-3β could be a common target for the SAFE and RISK pathways. In our model, both IPostC and pharmacological postconditioning with TNFα increase the phosphorylation of GSK-3β in WT mice. In contrast, we were surprised to observe that the absence of an infarct-sparing effect in TNF\textsuperscript{−/−} mice was associated with a similar level of Phospho/Total GSK-3β as that of WT mice subjected to the TNF-PostC protocol. Although our data support the proposal suggesting that the inactivation of GSK-3β is not an essential link in the cardioprotective effect of IPostC, this still remains controversial.\textsuperscript{31,34} Further studies using GSK-3β genetically modified mice will be required to confirm whether GSK-3β is or is not an effective downstream target of the TNFα/JAK/STAT-3 pathway.

Additional possible downstream targets of the SAFE pathway include phosphorylation of the pro-apoptotic target Bad\textsuperscript{14,29} and the inactivation of NFκB.\textsuperscript{7,35} Also, the inhibition of the mitochondrial permeability transition pore opening\textsuperscript{36} is likely to be the common point at which the SAFE and RISK pathways converge.

4.4 Other alternative pathways operative in postconditioning

The presence of multiple protective pathways has already been suggested in ischaemic preconditioning\textsuperscript{14,37} and it is likely that the SAFE and RISK pathways are activated in parallel with other paths in IPostC. Hence, sphingolipids have been proposed as members of a downstream activated path in IPostC.\textsuperscript{38,39} Sphingosine-1-phosphate can activate Akt, Erk1/2 and STAT-3 in the heart.\textsuperscript{39–41} Although TNFα protects via the activation of the sphingolipids,\textsuperscript{12} preliminary data in our laboratory suggest that sphingosine-1-phosphate mimics ischaemic preconditioning via the activation of STAT-3,\textsuperscript{42} the existence of sphingosine-1-phosphate as an alternative path to the SAFE or RISK pathway still needs to be confirmed (Figure 7).

4.5 Limitations to the study

The use of an isolated heart system does not take into account the intracoronary activation of leucocytes which may result in a detrimental effect of TNFα in the setting of myocardial ischaemia. Our data will need to be validated in vivo. Kinetic analysis of blood and cardiac TNFα levels following IPostC would facilitate the understanding of the exact role of TNFα as a cardioprotective agent against reperfusion injury. However, previous data exploring the protective effect of TNFα have demonstrated a good correlation between in vitro and in vivo preconditioning experiments, therefore suggesting that the circulating leucocytes do not play a major role.\textsuperscript{14,29} Alternatively, the cellular origin of the cardioprotective TNFα may reside in cardiomyocytes, and other cells such as resident cardiac mast cells and macrophages.\textsuperscript{43,44}

Another limitation to the study lies in the use of non-specific inhibitors, such as wort and AG490. Wort inhibits the PI-3 kinase signalling cascade and not Akt directly. AG490 blocks JAK2, which is upstream of STAT-3. Therefore, the use of such inhibitors can only indicate that a particular signalling cascade is blocked, but cannot pinpoint the exact downstream protein kinase involved. Further studies, either with siRNA techniques or genetically modified mice, such as
Akt null mice, may give greater specificity to the findings of the present study.

4.6 Conclusion

In summary, our studies have consistently shown that activation of the SAFE pathway, as represented by the activity of TNFα and STAT-3, is required in both ischaemic pre- and postconditioning.13–15,27,29,45 The specific involvement of TNFR2 in our studies suggests that this protective pathway is linked to this receptor. Overall, these results further emphasize the importance of RISK-independent pathways in cardiac protection at the time of reperfusion, which may have potential therapeutic application in the mitigation of ischaemic–reperfusion injury.

Supplementary material

Supplementary Material is available at Cardiovascular Research online.

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References


