Title: Protective efficacy of Toxoplasma gondii calcium-dependent protein kinase 1 (TgCDPK1) adjuvated with recombinant IL-15 and IL-21 against experimental toxoplasmosis in mice

Authors:

Jia Chen (chenjia_2009@163.com)
Si-Yang Huang (huangsiyang@caas.cn)
Zhong-Yuan Li (541929885@qq.com)
Eskild Petersen (joepeter@rm.dk)
Hui-Qun Song (songhuiqun@caas.cn)
Dong-Hui Zhou (donghui822002@163.com)
Xing-Quan Zhu (xingquanzhu1@hotmail.com)

Version: 2
Date: 1 August 2014

Author’s response to reviews: see over
Protective efficacy of *Toxoplasma gondii* calcium-dependent protein kinase 1 (TgCDPK1) adjuvated with recombinant IL-15 and IL-21 against experimental toxoplasmosis in mice

Jia Chen, Si-Yang Huang, Zhong-Yuan Li, Eskild Petersen, Hui-Qun Song, Dong-Hui Zhou and Xing-Quan Zhu

1 August 2014

Dr Cherrylyn Raytos
*BMC Infectious Diseases*

**Dear Dr Raytos,**

**Re: Revised Manuscript ID 6776659991211609**

On behalf of all co-authors, I would like to thank you and the two Reviewers very much for favorable and positive comments and constructive suggestions on our manuscript (MS) ID 6776659991211609. These comments and suggestions are very valuable for us to revise and improve the quality of our MS. The reviewers considered that our MS is of general interest to the readers of your esteemed journal *BMC Infectious Diseases*, and recommended the publication of our MS after revision. We have revised and improved the MS strictly according to the reviewers’ comments and suggestions. We used the “tracked changes” mode in the WORD to show the revised/changed text and sentences in the revised MS. Two MS files are uploaded: one shows “tracked changes” as “Additional material”, and the other is a “clean file” as “Main manuscript”. In the following, we detail our point-by-point responses to these specific comments and suggestions.

**Responses to comments and suggestions of Reviewer #1:**

**-Major Compulsory Revisions**

**Reviewers' general comments:**
This article deals with an important issue, i.e. control of *Toxoplasma gondii* infection by means of vaccination, since there is no effective treatment and prevention is difficult due to parasite’s promiscuity and presence of wild cycle. Nevertheless, there is one major issue which may preclude its publication and other important aspects that –given they properly answer the major one- must be addressed as well: The article contains several results which demonstrate specific response against *T. gondii* when they inject the adjuvant plasmid alone (i.e. that which codes for IL-21 and IL-15 only); these responses include antibody synthesis and antigen driven cytokine production and lymphocyte proliferation. This induction cannot be explained by the parasite challenge, since they find these responses before it. The decrease
in cyst burden or survival due to this adjuvant administration may be explained and is valuable, but the presence of the specific response might indicate a poor design, and thus all results would be questionable.

**Responses:** We thank Reviewer #1 very much for constructive comments and suggestions. Yes, the pVAX/mIL-21/mIL-15 group did work so well even when testing cytokine production and lymphocyte proliferation after stimulation by TLA, as well as antibody synthesis. In our previous study (Li et al., 2014), but also in this study, we have shown that in addition to the ability of improving TgMIC8-specific or TgCDPK1- immune responses, the administration of pVAX-IL-21-IL-15 alone could elicit a considerable non-specific protective immunity equivalent to the levels induced by pVAX-MIC8 or pVAX-CDPK1. Taken together, the synergy of IL-21 and IL-15 appears to have broad effect that could be used for the development of DNA vaccines against *T. gondii*. The effect of co-administration of rIL-15 and rIL-21 in absence of the antigen appeared to induce auto-immunity problems during our experiment, and we have observed clinical symptoms both in this study and our previous study (Li et al., 2014), which may be resulted from the high dose of co-injection of rIL-15 and rIL-21 to mice. Therefore, we should further investigate the optimal concentration of co-administrated rIL-15 and rIL-21 to model animals (e.g. mice) so as to induce effective acquired immune response but NO auto-immunity problems.

**Point 1:** There is an excess of information related to infection prevalence in different animal species and poor about the variety of vaccines actually being tested, both in acquired and congenital toxoplasmosis, so the background displayed in the introduction section is insufficient, and thus the “state of the art” is not explained to the reader. Although they comment about other vaccines in the Discussion, they should be mentioned in the introduction, and what made the authors to think in this new design.

**Responses:** We thank Reviewer #1 very much for constructive comments and suggestions on our MS. Immunoprophylaxis is one of key measures to control toxoplasmosis. So, an effective vaccine preventing infection in animals used for human consumption would block the main transmission route to humans (Zhang, et al., 2014; Innes, 2011). Although several types of vaccines have been developed including genetically engineering vaccines, subunit vaccines, especially, a live and attenuated vaccine of *T. gondii* S48 strain named ToxoVax has been licensed and used in farm animals, but it has limitations of poor efficacy or biosafety concerns (Zhang, et al., 2014; Innes, 2011). Most efforts have been made on DNA vaccines due to their capacity to induce a Th1-type immune response including a strong CD8+ cytotoxic T-lymphocyte (CTL) response (Gurunathan et al., 2000; Hoft et al., 2007; Cherif et al., 2011).

Moreover, TgCDPK1 is conserved among apicomplexans, involved in important biological functions, including the regulation of the parasite’s life cycle at stages dependent on microneme secretion, and it is recognized as the key regulator of calcium dependent exocytosis and acts in calcium-dependent secretion of specialized organelles called
micronemes, which play a critical role in direct parasite motility, host-cell invasion, and egress (Lourido et al., 2011), but also CDPKs have been identified in plants, ciliates and apicomplexans but not expressed by mammals, which represents validated target that may be exploitable for vaccine candidate against *T. gondii*. So, in this study we constructed a eukaryotic plasmid, pVAX-CDPK1, and examined the immunogenicity, and protective immune effect of this DNA vaccine in Kunming mice against *T. gondii* infection. Also, we have added these contents to the “Introduction” section in order to explain the “state of the art” to the reader sufficiently.

**Point 2:** Likewise, there is no information regarding the general immune profile which controls parasite proliferation, but until de Discussion and thus the use of the IL-15-IL-21 plasmid as adjuvant has no fundament. The role of these cytokines in toxoplasmosis and their relation to the “Th1/TH2” responses (which they determined as a measure of specific response) should be at least mentioned and referenced.

**Responses:** We thank Reviewer #1 very much for constructive comments and suggestions. In our previous studies, we have found that co-administration of IL-21 and IL-15 could be used as adjuvants and boost antigen-specific humoral as well as Th1 cellular immune responses induced by DNA vaccine against *T. gondii* infection (Li et al., 2014). Also, we have added these sentences to the “Introduction” section.

**Point 3:** The authors do not give details about the mouse strain. Are these animals inbred? Do they have resemblance to any known resistant or susceptible strains? This is relevant, since many -if not most- studies with which they compare their results, have been performed with known –mostly inbred- mice.

**Responses:** We thank Reviewer #1 very much for constructive comments and suggestions on our MS. The Kunming mice used in this study were inbred. It would be ideal to use international common laboratory mice (such as BALB/c, C57BL/6J), but these mice were not readily available in Lanzhou City, Gansu Province, China, a less-advanced region of China. Kunming mice are the most commonly used laboratory animals for biological and biochemical studies in China, and they are readily available. A number of previous studies have shown that Kunming mice are quite susceptible to *T. gondii* infection, and they serve as ideal model for vaccination studies against *T. gondii*. In this study we used Kunming mice as the laboratory animals, this allow us to compare the results of the present study with that of our previous studies also using Kunming mice as the laboratory animals (eg., Peng et al., 2009; Yuan et al., 2011; Wang et al., 2012; Yan et al., 2012; Chen et al, 2013; Yuan et al., 2013). We will strive to use international common laboratory mice (such as BALB/c, C57BL/6J) in our future vaccination studies against *T. gondii*.

**Point 4:** There is lack of statistical analysis test of the survival data; this should have been
done to assess difference between the group treated with the CDPK1-plasmid and that treated with both plasmids, so they could sustain the combined effect.

**Responses:** We thank Reviewer #1 very much for constructive comments and suggestions. In this study, there was significant difference in survival time between the group of pVAX-CDPK1 and that of pVAX/IL-21/IL-15 ($P < 0.05$). Co-administration with pVAX-CDPK1 (17.3 ± 4.3 days) and pVAX/IL-21/IL-15 (12.0 ± 2.0 days) enhanced the survival time of the immunized mice (19.2 ± 5.1 days), in contrast to the group of pVAX-CDPK1 or pVAX/IL-21/IL-15 ($P < 0.05$). Also, we have added the statistical analysis test of the survival data in the section “Assessment of protective efficacy of DNA immunized mice against *T. gondii*” in the “Results” section.

**Point 5:** As mentioned, it is unclear why they obtain *T. gondii* specific antibodies (figure1), CD pattern, proliferation and cytokine production (table 1 and figure 2) when they inoculate the plasmid with the adjuvant cytokines alone (pVAX/IL21/IL15). Moreover, most results are equal between plasmid containing specific CDPK1 alone and IL21/IL15 alone, and when there has not been challenge infection.

**Responses:** We thank Reviewer #1 very much for constructive comments and suggestions on our MS. In our published paper (Li, et al., 2014), we have pointed out “IL-15 induces proliferation and cytokine production in T- and NK cells [45,46]. IL-21 is a T cell derived cytokine that works in synergy with other cytokines and in synergy with IL-7 and IL-15 activate and expand CD8+ cytotoxic T cells [47]. Unlike interleukin-2, IL-15 protects from activation-induced cell death and does not promote regulatory cells. IL-21 stimulates the proliferation of CD4+and CD8+ T lymphocytes and regulates the profile of cytokines secreted by these cells, drives the differentiation of B cells into memory cells and Ig-secreting plasma cells, and enhances the activity of natural killer cells [48]. Therefore, it is not surprising to see an unspecific immunoprotective response from the adjuvants alone because of an unspecific activation of CD8+ cytotoxic T cells”. So, we have chosen pVAX/mIL-21/mIL-15 as an adjuvant in pVAX-CDPK1 DNA vaccine in the present study. As results, we also found that the administration of pVAX-IL-21-IL-15 alone elicited a considerable non-specific protective immunity equivalent with the levels induced by pVAX-CDPK1, which emphasized again that cytokine adjuvant pVAX-IL-21-IL-15 could act as immunotherapeutic modulation used for *T. gondii* vaccines [Li et al., 2014].

**Point 6:** It is also unclear why they tested IgG subclass determination at the second week, when the IgG response is suboptimal if it is compared with those found at weeks 4 or 6 (see figure 1).

**Responses:** We thank Reviewer #1 very much for constructive comments and suggestions on our MS. In this study, we do have tested IgG subclass including IgG1 and IgG 2a two weeks after the last immunization (at the six weeks), while the IgG response is optimal as shown in Figure 1A and Figure 1B. Then, the increase of total antibody IgG levels occurred with
successive DNA immunizations (Figure 1A).

**Point 7:** Results on survival (figure 3) are the only which suggest CDPK1 is protective, but apparently it is so even without the need of the IL21/IL15 plasmid effect, unless proper statistics shows otherwise (see above).

**Responses:** We thank Reviewer #1 very much for constructive comments and suggestions on our MS. We have added the “A, B, C and D” to the Figure 3, in which the same letter in front of experimental group means no statistically significant difference (P > 0.05) between different experimental groups from the same measurement, while different letter means statistically significant difference (P < 0.05). Also, we have revised the Figure legend of Figure 3.

**Point 8:** Although the Discussion addresses several aspects not introduced in the first part of the paper, their vaccine design should be more detailed compared to others (actually many) of the literature, both in terms of design and results. It would seem that a combined vaccine needs to be evaluated; so the question remains as to why they developed a new, single one and why they used the IL-21/IL-15-plasmid.

**Responses:** We thank Reviewer #1 very much for constructive comments and suggestions on our MS. We have improved the “Discussion” section in order to explain why we developed a new, single one and why we used the IL-21/IL-15-plasmid.

- Minor Essential Revisions

**Point 1:** The last sentence of the introduction lacks text within “..and increase the of protective *T. gondii* immunity.”

**Responses:** We thank Reviewer #1 very much for constructive comments and suggestions on our MS. We have revised this sentence accordingly.

**Point 2:** It is unclear in the Methods section what were 10 mice used for: in page 5, it is stated that they used 35 mice per group; then in page 5 challenge with the RH and PRU strains are described for 15 and 10 mice respectively; thus, there are 10 animals not described, or so it seems.

**Responses:** We thank Reviewer #1 very much for constructive comments and suggestions on our MS. In our study, we used a total of 35 mice per group, including 15 mice used for challenge with the RH strain, 10 mice used for challenge with PRU strain, and 10 mice used for lymphocyte proliferation assays, cell surface staining of splenic lymphocytes, cytokine assays and antibody analysis.

**Point 3:** In the Results section, the text “but there was not any significant different between three control groups….” could be “but there was no significant difference among the three control groups….”
Responses: Revised accordingly.

Point 4: In the Discussion: “The results showed that immunization intramuscularly with…” could be “The results showed that intramuscular immunization with…”
Responses: Revised accordingly.

Level of interest: An article of importance in its field
Responses: We thank Reviewer #1 very much for his favorable and positive comments.

Quality of written English: Needs some language corrections before being Published.
Responses: We thank Reviewer #1 very much for his favorable and positive comments. We have improved the English language of the MS accordingly.

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Responses: We thank Reviewer #1 very much for constructive comments. We have made sure that the statistical analysis used for this study is properly, and the statistical data can reflect the results apparently.

Responses to comments and suggestions of Reviewer #2:

- Discretionary Revisions
Point 1: I am unfamiliar with Kunming mice. A brief description of these mice and why they were chosen for use in the experiments could be provided.
Responses: We thank Reviewer #2 very much for constructive comments and suggestions on our MS. The Kunming mice used in this study were inbred.

It would be ideal to use international common laboratory mice (such as BALB/c, C57BL/6J), but these mice were not readily available in Lanzhou City, Gansu Province, China, a less-advanced region of China. Kunming mice are the most commonly used laboratory animals for biological and biochemical studies in China, and they are readily available. A number of previous studies have shown that Kunming mice are quite susceptible to T. gondii infection, and they serve as ideal model for vaccination studies against T. gondii infection.

In this study we used Kunming mice as the laboratory animals, this allow us to compare the results of the present study with that of our previous studies also using Kunming mice as the laboratory animals (eg., Peng et al., 2009; Yuan et al., 2011; Wang et al., 2012; Yan et al., 2012; Chen et al, 2013; Yuan et al., 2013). We will strive to use international common laboratory mice (such as BALB/c, C57BL/6J) in our future vaccination studies against T. gondii.

Point 2: Authors mention in discussion that CDPK1 is conserved between different strains of T. gondii, but is it expressed in the bradyzoite as well as the tachyzoite stage?
Responses: We thank Reviewer #2 very much for constructive comments and suggestions on our MS. Yes, CDPK1 is expressed in the bradyzoite as well as the tachyzoite stage. Also, we have added this sentence to the “Discussion” section, in order to explain the “…CDPK1 is conserved between different strains of T. gondii” to the reader sufficiently.

- Minor Revisions

Point 1: One of the headings in the Methods section is “Expression of pVAX-IF2a plasmid in vitro” however, this is not the protein used for the vaccination studies in this manuscript. This needs to be corrected.

Responses: We thank Reviewer #2 very much for excellent comments. We are very sorry we made a silly mistake. We have revised the “Expression of pVAX-IF2a plasmid in vitro” to “Expression of pVAX-CDPK1 plasmid in vitro”

Point 2: Authors state (page 6) that they vaccinated intramuscularly twice at 2-week intervals, but then list three time points. Clarify is it two or three immunizations?

Responses: We thank Reviewer #2 very much for constructive comments and suggestions on our MS. In our study, the animals were intramuscularly injected twice at 2-week intervals in three immunizations (at weeks 0, 2 and 4). Also, we have revised the sentences accordingly in the text.

Point 3: In reference to Table I the authors mention there was not any significant difference between three control groups, but only two control groups are found in the table.

Responses: We thank Reviewer #2 very much for constructive comments and suggestions on our MS. We are very sorry we made a mistake here. In reference to Table 1, three control groups included PBS, control and pVAX I. Also, we have replaced the “Table 1” by a new one.

Point 4: For figure 2 which control group is shown in the figure? They mention three control groups were used in this experiment.

Responses: We thank Reviewer #2 very much for constructive comments and suggestions on our MS. For Figure 2, the blank control group is shown in the figure. Since no significant differences between those three control groups, we have not shown the results of the other two control groups including pVAX I and PBS so as to simplify the Figure 2.

Point 5: Authors could draw upon more primary source material and not almost exclusively reference reviews, with the exception of their own work, throughout the paper.

Responses: We thank Reviewer #2 very much for constructive comments and suggestions on our MS. We have revised the content referred to our published paper accordingly in the “materials and methods”.
Point 6: Table 1 needs clarity. Do the different superscript letters refer to a difference in significance compared to the PBS control or between other groups?

**Responses:** We thank **Reviewer #2** very much his constructive comments and suggestions on our MS. In “Table 1”, the same superscript letter on the shoulders of experimental data means no statistically significant difference (P > 0.05) between different experimental groups from the same measurement, while different letter means statistically significant difference (P < 0.05). Also, we have revised the relevant sentences in the “Table 1”, so as to clarify the significant difference of statistical data.

Point 7: In figure 1 the authors indicate there is a significant difference in IgG in the serum after pVAX/CDPK1 and pVAX/IL-21/IL-15 vaccination at 2 and 4 weeks after initiation of vaccination; however, looking at the data I don’t see how this is possible. Are the authors instead trying to indicate a significant difference between pVAX/IL-21/IL-15 and pVAX/CDPK1 + pVAX/IL-21IL-15 immunization?

**Responses:** We thank **Reviewer #2** very much for constructive comments and suggestions on our MS. Yes, we are sure that there is a significant difference in IgG in the serum after pVAX/CDPK1 and pVAX/IL-21/IL-15 vaccination at 2 and 4 weeks after initiation of vaccination by statistical analysis. In the meanwhile, pVAX/CDPK1 + pVAX/IL-21IL-15 immunization have induced higher level of IgG than that in both groups of pVAX/CDPK1 and pVAX/IL-21/IL-15.

- **Major Compulsory Revisions**

Point 1: There are a number of typos, spelling errors and grammatical errors throughout the manuscript that need to be corrected before resubmission. Also, the authors have paragraphs that are only one sentence in length (paragraph 3 in discussion).

**Responses:** We are very grateful to **Reviewer #2** for favorable comments and constructive suggestions on our MS. We have improved the English grammar and sentence structure of the MS accordingly.

Point 2: The authors need to provide stronger rationale as to why the TgCDPK1 protein would make a good vaccination candidate. Is this protein unique to this protozoan spp. and not expressed by mammals? Are serum Abs specific for this protein detected in infected humans and animals? Just because the protein is involved in motility and egress does not mean it will induce a protective Ab response upon challenge infection.

**Responses:** We are very grateful to **Reviewer #2** for favorable comments and constructive suggestions on our MS. TgCDPK1 is conserved among apicomplexans, involved in important biological functions, including the regulation of the parasite’s life cycle at stages dependent on microneme secretion, and it is recognized as the key regulator of calcium dependent exocytosis and acts in calcium-dependent secretion of specialized organelles called micronemes, which play a critical role in direct parasite motility, host-cell invasion, and
egress (Lourido et al., 2010), but also CDPKs have been identified in plants, ciliates and apicomplexans but not expressed by mammals, which represents validated target that may be exploitable for vaccine candidate against *T. gondii*. Also, we have revised the relevant content in the “Introduction” section.

**Point 3:** The expression of TgCDPK1 in the Marc-145 cells should be shown and control non-transfected Marc-145 cells should also be shown to confirm the specificity of the TgCDPK1 stain. This data is important because it helps address my concern in point #2, as the serum derived from the goat infected or immunized with *T. gondii* (specify) must contain antibodies specific for TgCDPK1 if it is able to stain cells transfected with the plasmid DNA. Thus, suggesting that animals infected naturally with *T. gondii* may make Abs against this protein.

**Responses:** We are very grateful to Reviewer #2 for favorable comments and constructive suggestions on our MS. Sorry, we made a mistake here. Also, we have provided a new figure, which have been presented as supplementary Figure 1 accordingly.

**Point 4:** The authors need to include rationale in the various sections within the results explaining why they are evaluating certain aspects of the immune response after vaccination.

**Responses:** We are very grateful to Reviewer #2 for favorable comments and constructive suggestions on our MS. We have added rationale in the various sections within the results in order to explain why we have evaluated certain aspects of the immune response after vaccination.

**Point 5:** In figure 1 the authors are only measuring total serum IgG or IgG1/IgG2c Ab levels after vaccination. However, this should be complimented with TgPDCK1-specific ELISAs primarily, because vaccination with the IL-15/IL-21 plasmid induced IgG production by itself and these Abs are not specific for CDPK1. Therefore, the majority of the Ab produced with the pVAX/CDPK1 + pVAX/IL-21/IL-15 co-vaccination could also be primarily non-specific IgG Ab, or it could in fact be specific for CDPK1.

**Responses:** We thank Reviewer #2 very much for constructive comments and suggestions on our MS. In our study, we used goat anti-mouse Ig capture (provided by commercial kit) antibody to capture total antibodies including IgG, IgG1 and IgG2a in serum. Following addition to HRP-labeled goat anti-mouse Ig screening antibody, HRP-labeled goat anti-mouse IgG1, HRP-labeled goat anti-mouse IgG2a, respectively, the levels of total antibodies, or IgG1 and IgG2a antibodies in serum samples were measured at 405 nm after substrate addition using ELISA reader. On the other hand, from these procedures, it has been suggested that Fig. 1 can not represent the total IgG clearly, but the results of IgG1 and IgG2a antibodies assay is another evidence for the increasing of IgG indirectly. We have revised some descriptions of Figure 1 accordingly in the “Humoral immune responses induced by DNA immunization” in “Results”.

9
**Point 6:** The conclusions the authors are trying to make in the discussion section are often not expressed with any clarity to the reader. For example, I’m not sure what point the author is trying to make in paragraph three. Why would the activation of a Th1 response by the vaccine prevent severe immunopathology during acute or chronic *T. gondii* infection? The authors never show this in the manuscript.

**Responses:** We thank **Reviewer #2** very much for constructive comments and suggestions on our MS. In this study, both IFN-γ and IL-2 secretion were significantly increased in splenocytes from pVAX-CDPK1 immunized mice and the low levels of IL-4 and IL-10 was also produced in contrast with the controls, demonstrating a Th1-biased cellular immune response, but also emphasized again the activation of an appropriate T helper response associated with high levels of Th1 and low levels of Th2 type cytokines could be contributed to prevent CD4+ T cell-mediated severe immunopathology during the acute and chronic stage of *T. gondii* invasion. Also, we have revised the relevant content in the “Discussion” section.

**Point 7:** In the discussion the authors should elaborate/speculate on the function of IL-15 and IL-21 during vaccination as well as reference their previous work and findings using this cytokine expression plasmid (Li et al., Vaccine 2014). What are the normal functions of these cytokines in an immune response? What cell types are they acting on after vaccination?

**Responses:** We thank **Reviewer #2** very much for excellent comments. In our previous studies (Li et al., Vaccine 2014), we have found that the synergy of rIL-15 and rIL-21 genes could augment the efficacy of DNA vaccine in the induction of Th1-biased response, and thus pVAX/mIL-21/mIL-15 alone could induced stronger immune responses resulting in further protective efficacy. So, we choose pVAX/mIL-21/mIL-15 as adjuvant in pVAX-CDPK1 DNA vaccine in the present study. Also, we have added the relevant content in the “Discussion” section.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Responses:** We thank **Reviewer #2** very much for favorable and positive comments.

**Quality of written English:** Not suitable for publication unless extensively edited.

**Responses:** We thank **Reviewer #2** very much for his constructive comments and suggestions. We have improved the English language of the MS accordingly.

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Responses:** We thank **Reviewer #2** very much for favorable and positive comments.

We sincerely hope that the MS has been revised to your satisfaction. We are looking forward
to seeing the acceptance of the revised MS for publication in your esteemed journal *BMC Infectious Diseases*.

With best wishes,

Xing
Xing-Quan Zhu, BVSc, MVSc, PhD
Professor and Head, Department of Parasitology,
Deputy-Director, State Key Laboratory of Veterinary Etiological Biology,
Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences,
1 Xujiaping, Yanchangbu, Lanzhou, Gansu Province 730046,
The People's Republic of China
Email: xingquanzh@scau.edu.cn; xingquanzhu1@hotmail.com
Tel: +86-931-8342837; Fax: +86-931-8340977